

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95

#3

FIG. 1A

1 agggagaggc agtgaccatg aaggctgtgc tgcttgcacct gtttatggca
51 ggcttggccc tgcagccagg cactgcacctg ctgtgtact cctgcaaagc
101 ccaggtgagc aacgaggact gcctgcaggt ggagaactgc acccagctgg
151 gggagcagtg ctggaccgcg cgcattccgcg cagttggcct cctgaccgtc
201 atcagcaaag gctgcagctt gaactgcgtg gatgactcac aggactacta
251 cgtggcaag aagaacatca cgtgcgtgtga caccgacttg tgcaacgcca
301 gcggggccca tgccctgcag ccggctgcgc ccatcatttc gctgctccct
351 gcactcgccc tgctgctctg gggaccgcgc cagctatagg ctctgggggg
401 ccccgctgca gcccacactg ggtgtggtgc cccaggcctt tgtgccactc
451 ctcacagaac ctggcccaagt gggaggcctgt cctggttccct gaggcacatc
501 ctaacgcaag tttgaccatg tatgtttgca cccctttcc cnnaaccctg
551 accttccat ggcccttttc caggattccn accnggcaga tcagtttag
601 tganacanat ccgcntgcag atggccccctc caaccnttn tgggttgtt
651 tccatggccc agcattttcc acccttaacc ctgtgttcag gcactnttc
701 ccccaggaag ccttccctgc ccacccatt tatgaattga gccaggttg
751 gtcgtggtg tccccgcac ccagcagggg acaggcaatc aggaggccc
801 agtaaaggct gagatgaagt ggactgagta gaactggagg acaagagttg
851 acgtgagttc ctggagttt ccagagatgg ggcctggagg cctggaggaa
901 gggccaggc ctcacatttgc tgggntccc gaatggcagc ctgagcacag
951 cgtaggccct taataaacac ctgttggata agccaaaaaaa aaaaaaaaa

FIG. 1B

MKAVLLALLMAGLALQPGTALLCYSCKAQVSNECLQV
ENCTQLGEQCWTARIRAVGLLTVISKGCSLNCVDDS
QDYYVGKKNITCCDTDLCNASGAHALQPAAAILALLPAL
GLLLWGPQQL

FIG. 2

ATGAAGACAGTTTTTTTATCCTGCTGGCCACCTACTTAGCCCTGCATCCAGGTGCTGCT
1 -----+-----+-----+-----+-----+-----+ 60
TACTTCTGTCAAAAAAAATAGGACGACCGGTGGATGAATGGGACGTAGGTCCACGACGA

M K T V F F I L L A T Y L A L H P G A A

CTGCAGTGCTATTCATGCACAGCACAGATGAACAAACAGAGACTGTCTGAATGTACAGAAC
61 -----+-----+-----+-----+-----+-----+ 120
GACGTCACGATAAGTACGTGTCGTCTACTTGTGCTCTGACAGACTTACATGTCTTG

L Q C Y S C T A Q M N N R D C L N V Q N

TGCAGCCTGGACCAGCACAGTTGCTTACATCGCGATCCGGGCCATTGGACTCGTGACA
121 -----+-----+-----+-----+-----+-----+ 180
ACGTCGGACCTGGTCGTCAACGAAATGTAGCGCGTAGGCCCGTAACCTGAGCACTGT

C S L D Q H S C F T S R I R A I G L V T

GTTATCAGTAAGGGCTGCAGCTCACAGTGTGAGGATGACTCGGAGAACTACTATTGGC
181 -----+-----+-----+-----+-----+-----+ 240
CAATAGTCATTCCCACGTCGAGTGTACACTCCTACTGAGCCTCTTGATGATAAACCG

V I S K G C S S Q C E D D S E N Y Y L G

AAGAAGAACATCACGTGCTGCTACTCTGACCTGTGCAATGTCAACGGGGCCCACACCTG
241 -----+-----+-----+-----+-----+-----+ 300
TTCTTCTTGTAGTGCACGACGATGAGACTGGACACGTTACAGTTGCCCGGGTGTGGGAC

K K N I T C C Y S D L C N V N G A H T L

AAGCCACCCACCACCTGGGGCTGCTGACCGTGTCTGCAGCCTGTTGCTGTGGGCTCC
301 -----+-----+-----+-----+-----+-----+ 360
TTCGGTGGGTGGTGGGACCCCGACGACTGGCACGAGACGTCGGACAACGACACCCGAGG

K P P T T L G L L T V L C S L L L W G S

AGCCGTCTGTAGGCTCTGGAGAGCCTACCATAGCCCATTGTGAAGGGATGAGCTGCAC
361 -----+-----+-----+-----+-----+-----+ 420
TCGGCAGACATCCGAGACCCCTCTGGATGGTATGGGCTAACACTTCCCTACTGACGTG

S R L *

TCCACCCCACCCCCACACAGG
421 -----+-----+-----+ 441
AGGTGGGGTGGGGGTGTGTCC

FIG. 3

1	M K I F L P V L L A A L L G V E R A S S	hSCA-2
1	M K A V L L A L L M A G L A L Q P G T A	hPSCA
1	M K T V L F L L A T Y L A L H P G A A	mPSCA
21	L M C F S G L N Q K S N * L Y C L K P T I	
21	L L C Y S C K A Q V S N * E D C L Q V E N *	
21	L Q C Y S C T A Q M N N * R D C L N V Q N *	
41	C S D Q D N Y C V T V S A S A G I G N L	
41	C T Q L G E Q C W T A R I R A V G G L L T	
41	C S L D Q H S C F T S R I R A I G L V T	
61	V T F G H S L S K T G S P A C P I P E G	
61	V - - - - I S K G C S L N G V D D S Q	
61	V - - - - I S K G C S S Q C E D D S E	
81	V' N V G V A S M G I S C C Q S F L C N * F	
76	D Y Y V G K K - N * I T C C D T D L C N * A	
76	N Y Y L G K K - N * I T C C Y S D L C N * V	
101	S A A D G G L R A S V T L I G A G L L L	
95	S G A H A L Q P A A A I L A L L P A L G	
95	N G A H T L K P P T T L G L L T V L C S	
121	S L L P A L L R F G P	
115	L L L W G P G Q L - -	
115	L L L W G S S R L - -	

FIG. 4

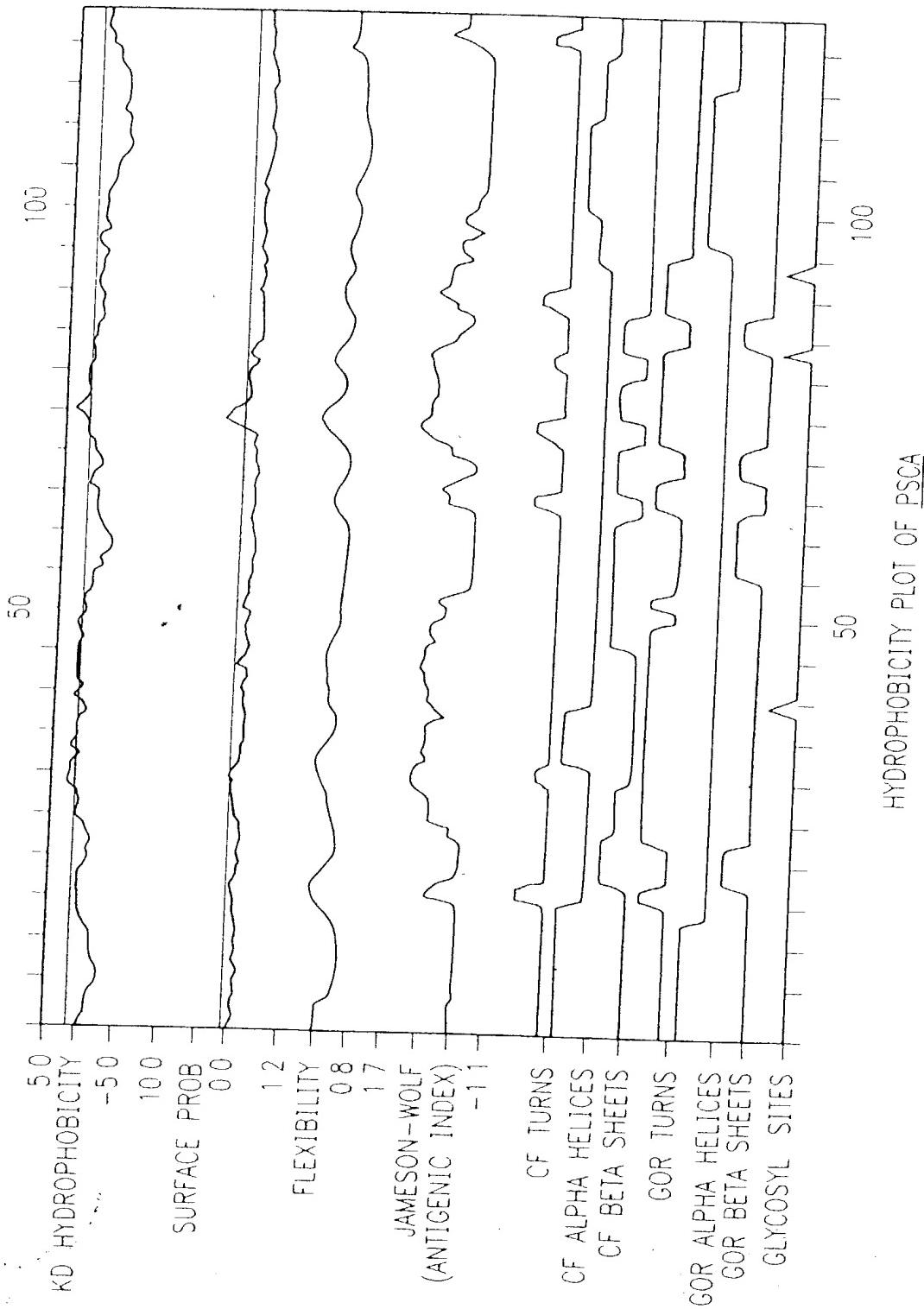


FIG. 5

GPI SIGNAL
SIGNAL SEQUENCE
/ = glycosylation SITE

LAPC9
S. INTESTINE
TESTIS
KIDNEY
KIDNEY
BLADDER CARCINOMA
BLADDER
BLADDER
PROSTATE
PROSTATE
PROSTATE



FIG. 6

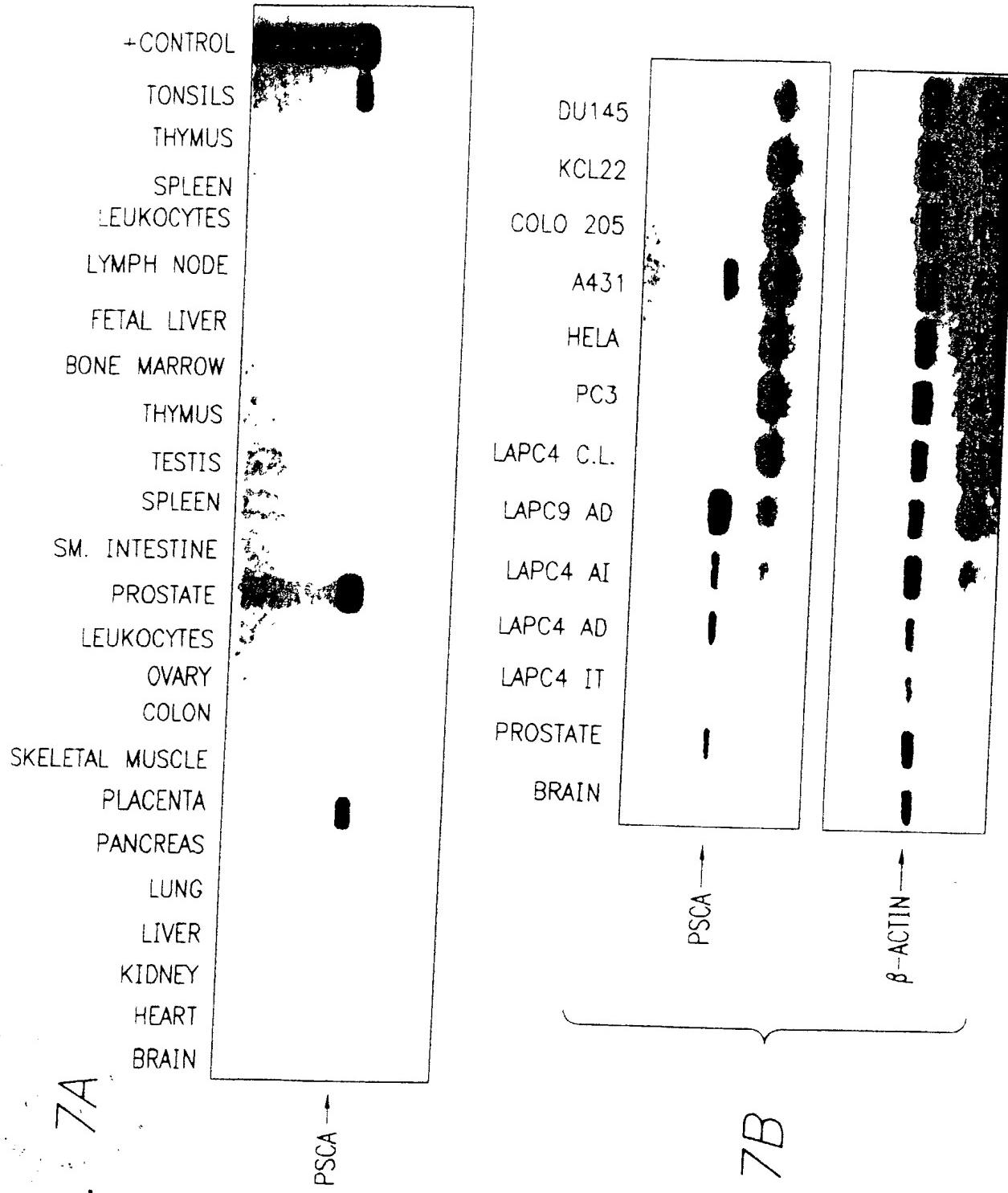


FIG. 8A

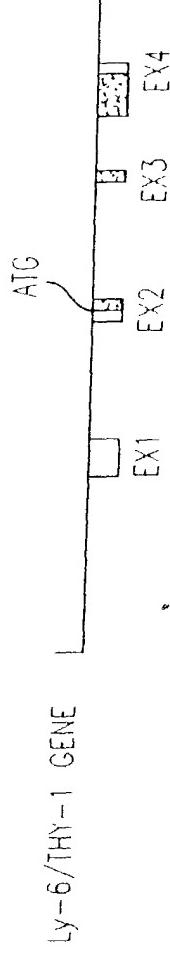


FIG. 8B

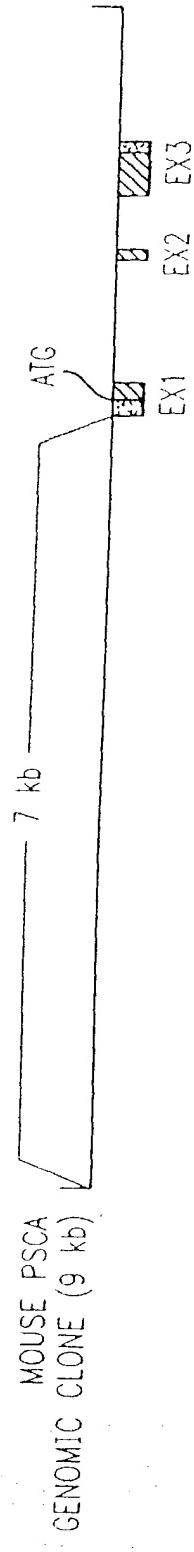


FIG. 8C

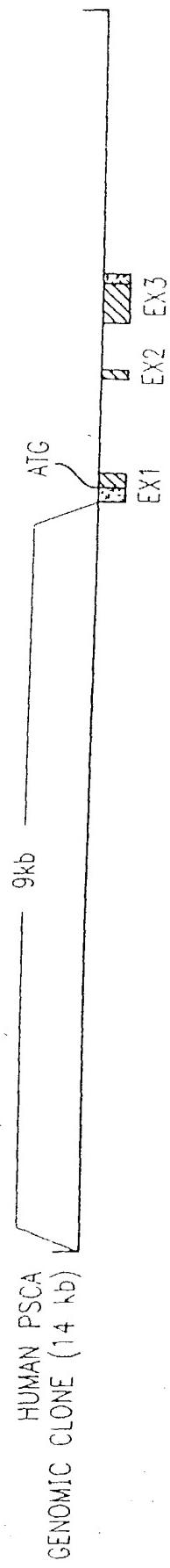


FIG. 9A

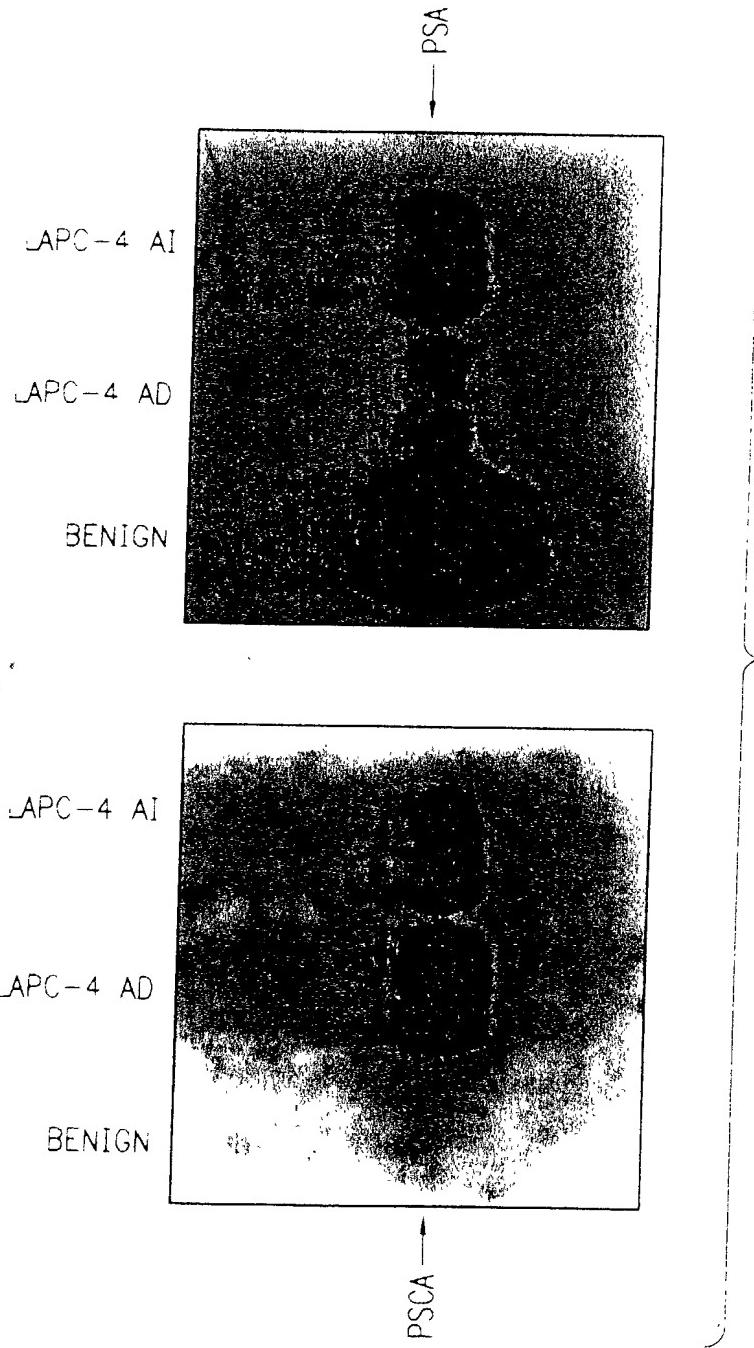
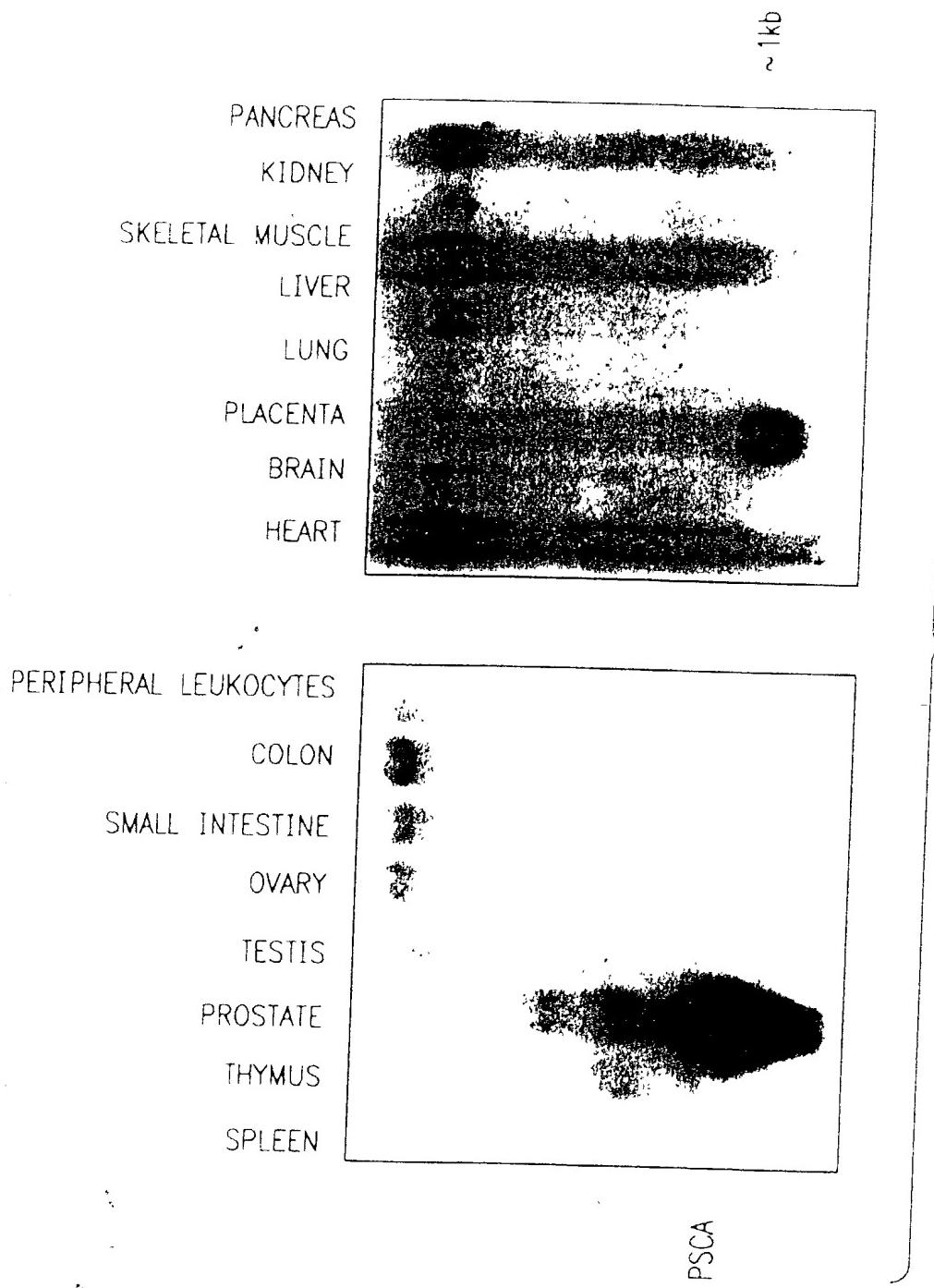
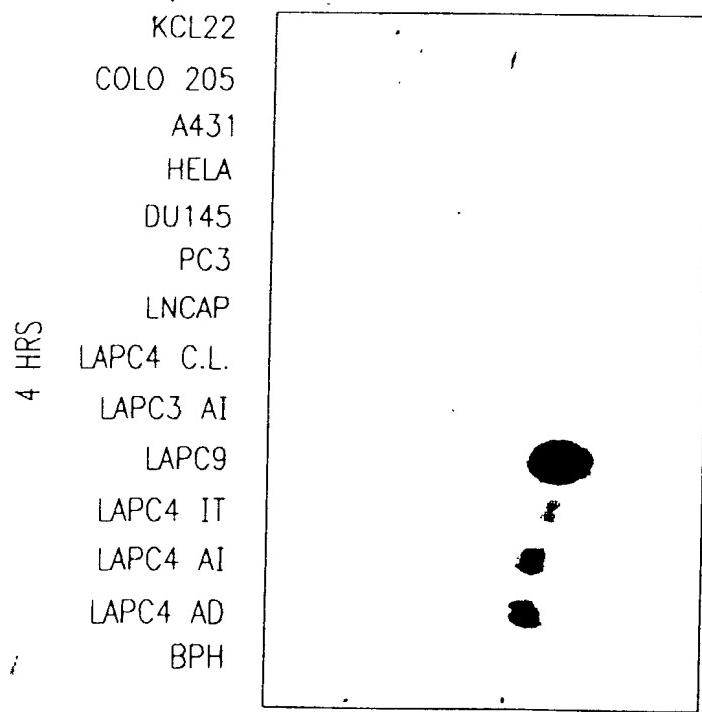
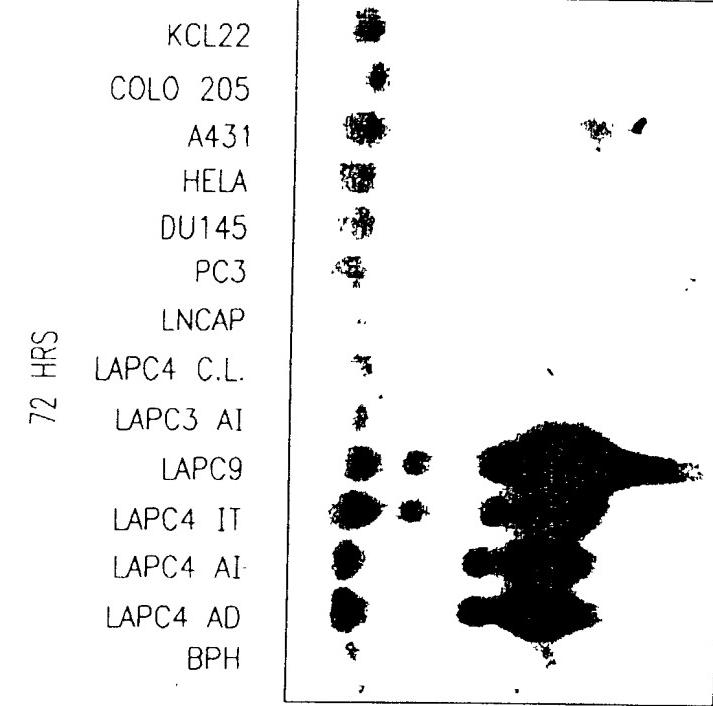


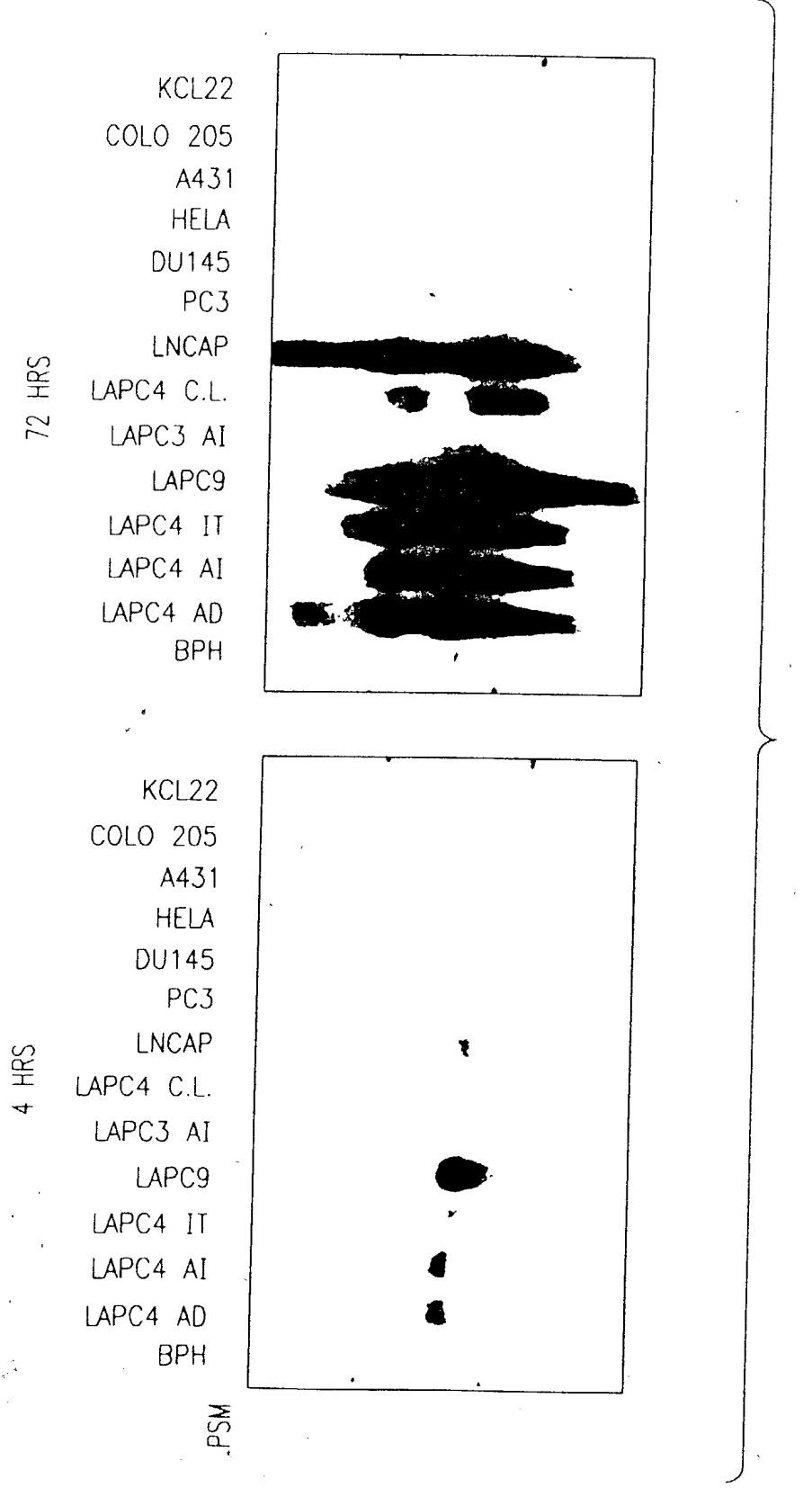
FIG. 9B





PSCA

FIG. 10A



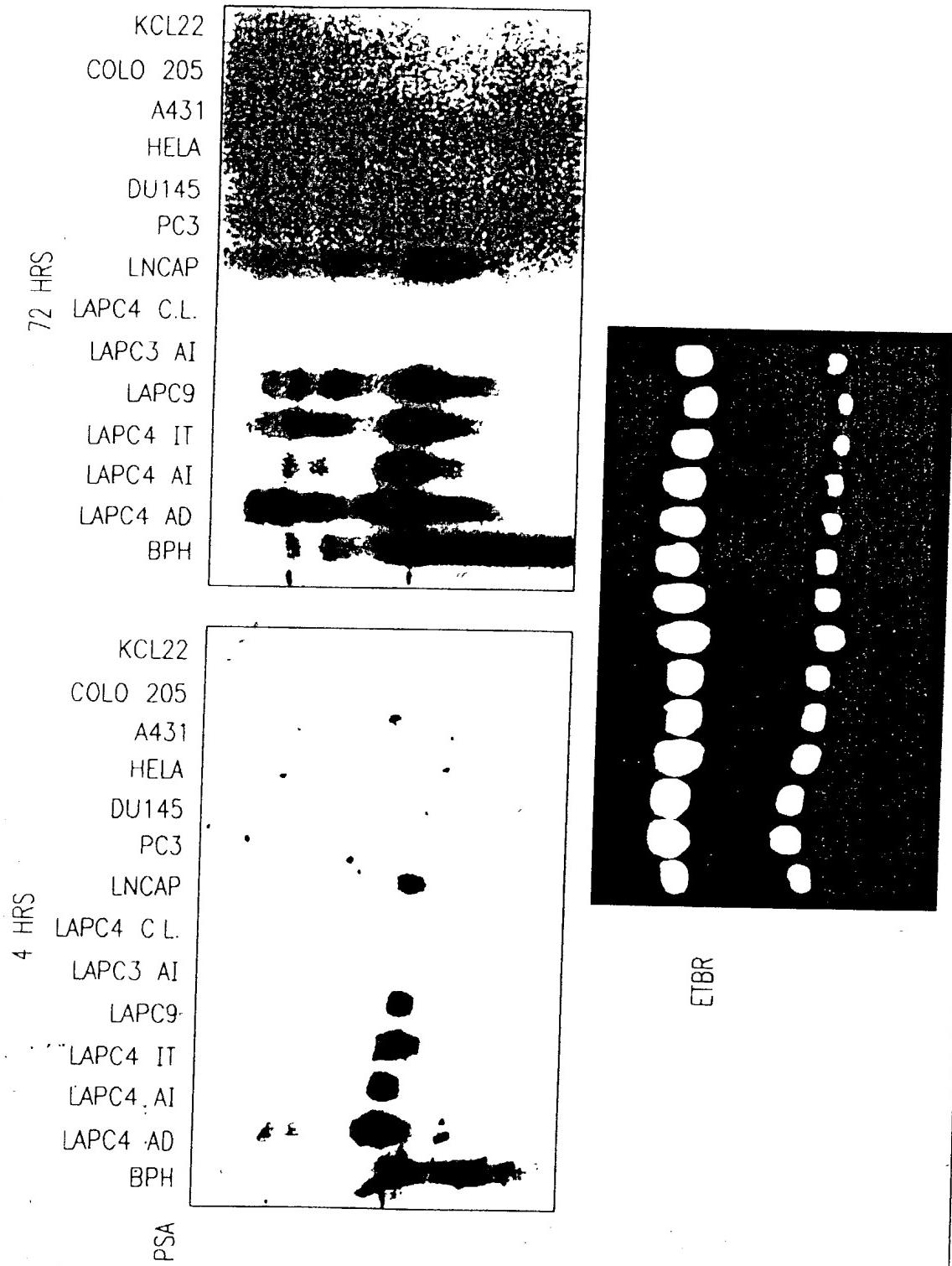


FIG. 10C

FIG. 11A

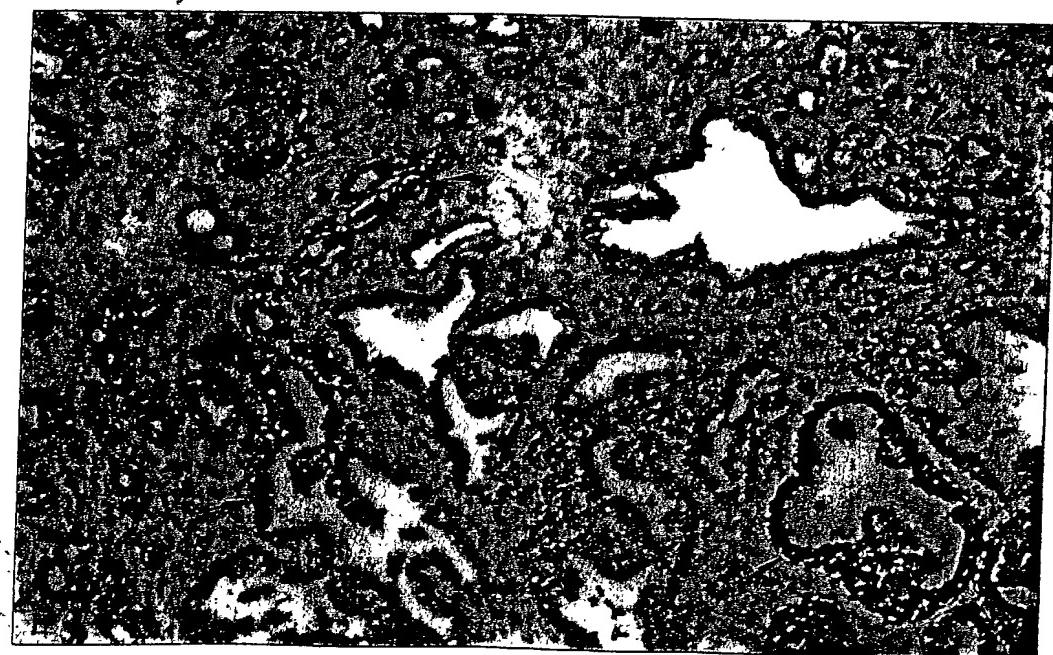


FIG. 11B

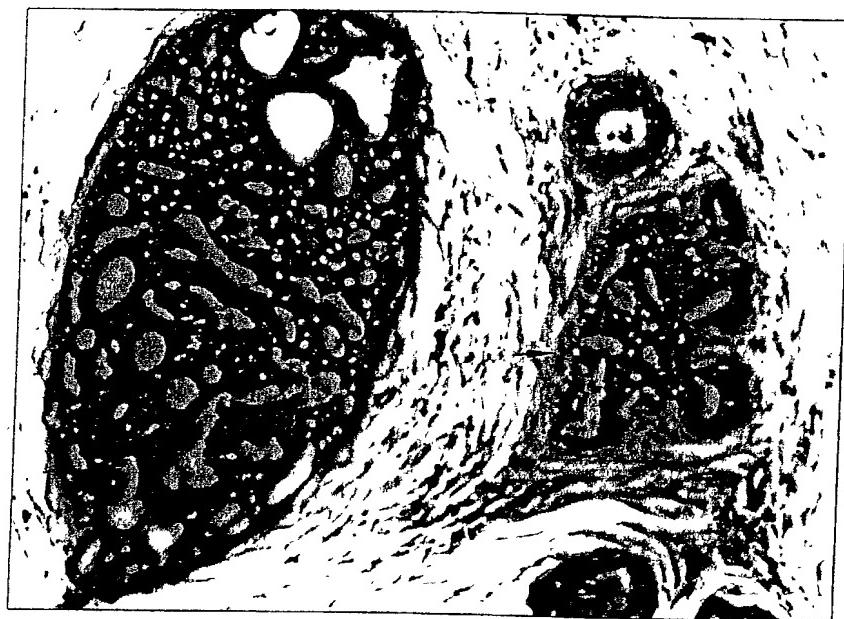


FIG. 11C

FIG. 1.2A

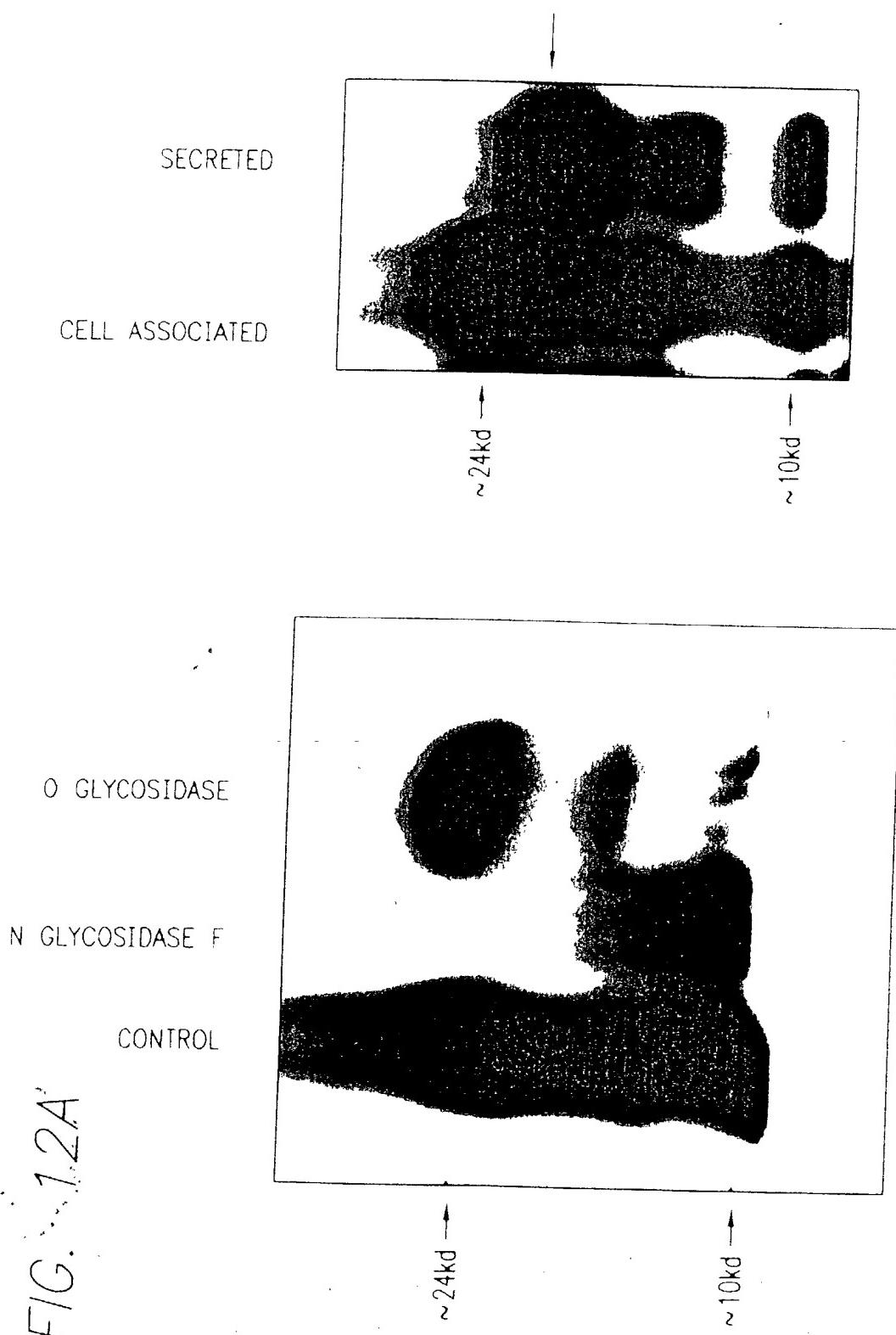
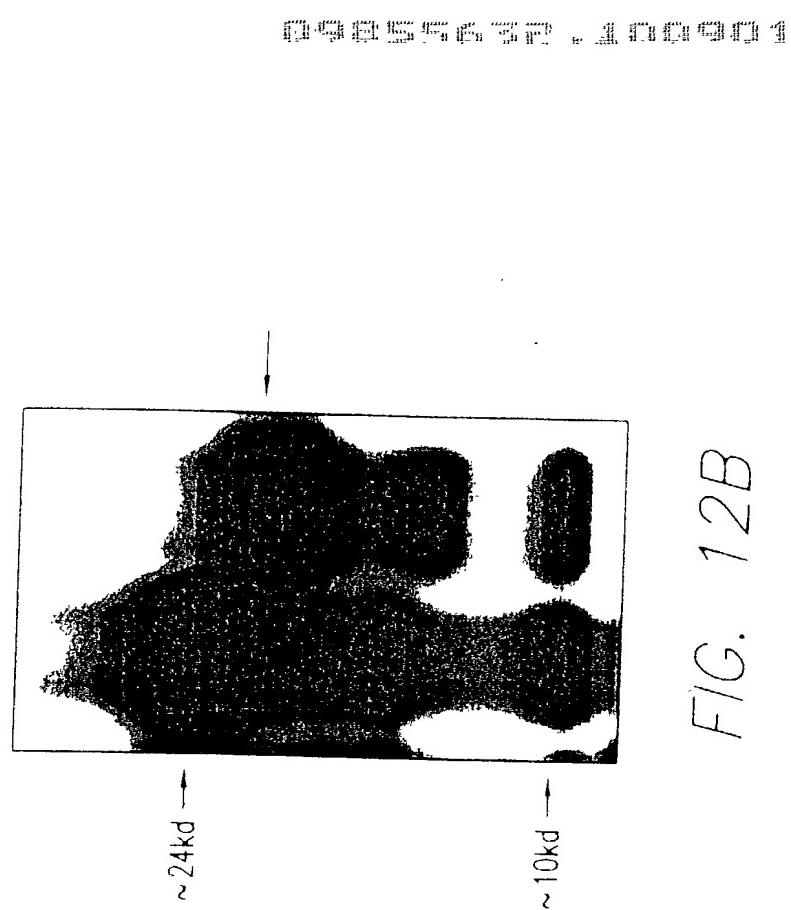


FIG. 1.2B



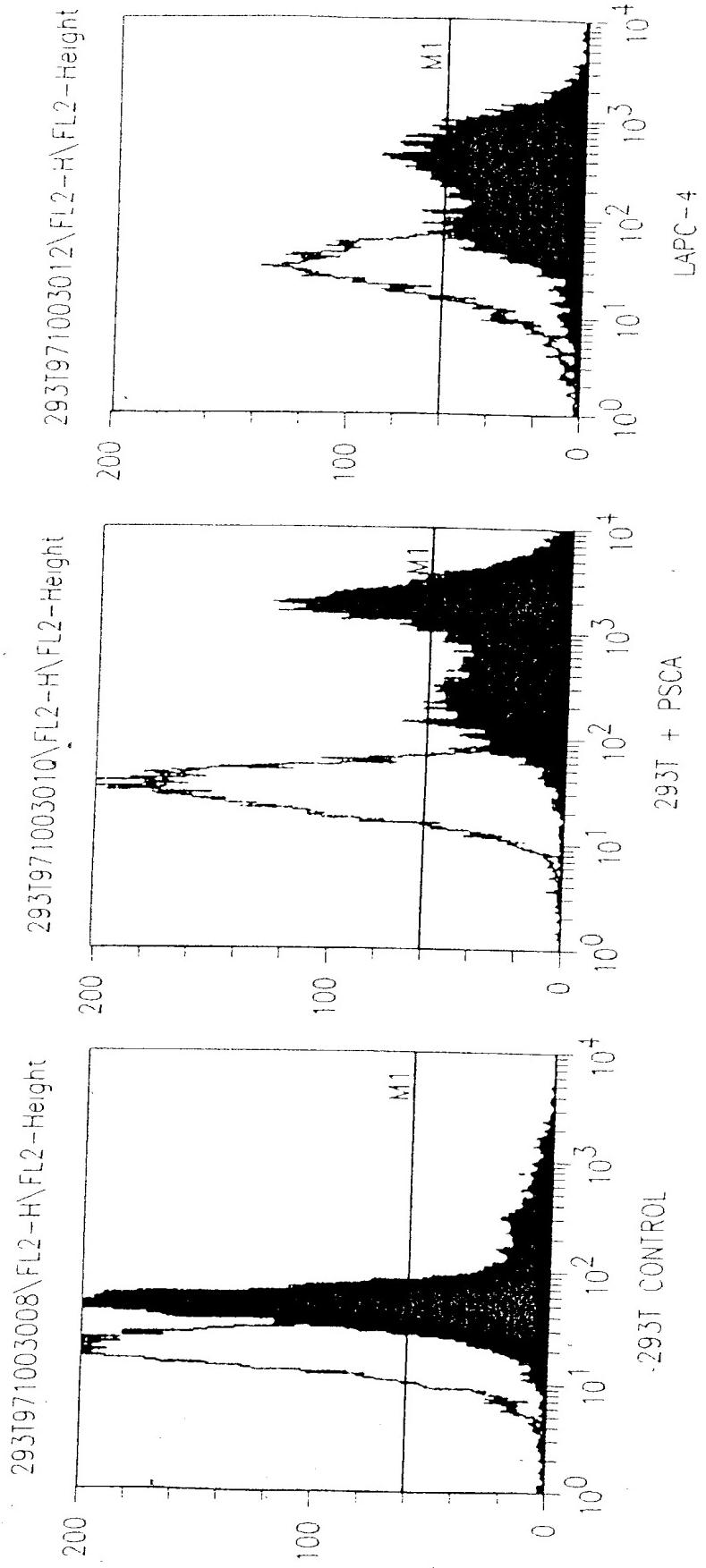


FIG. 12C

FIG. 13

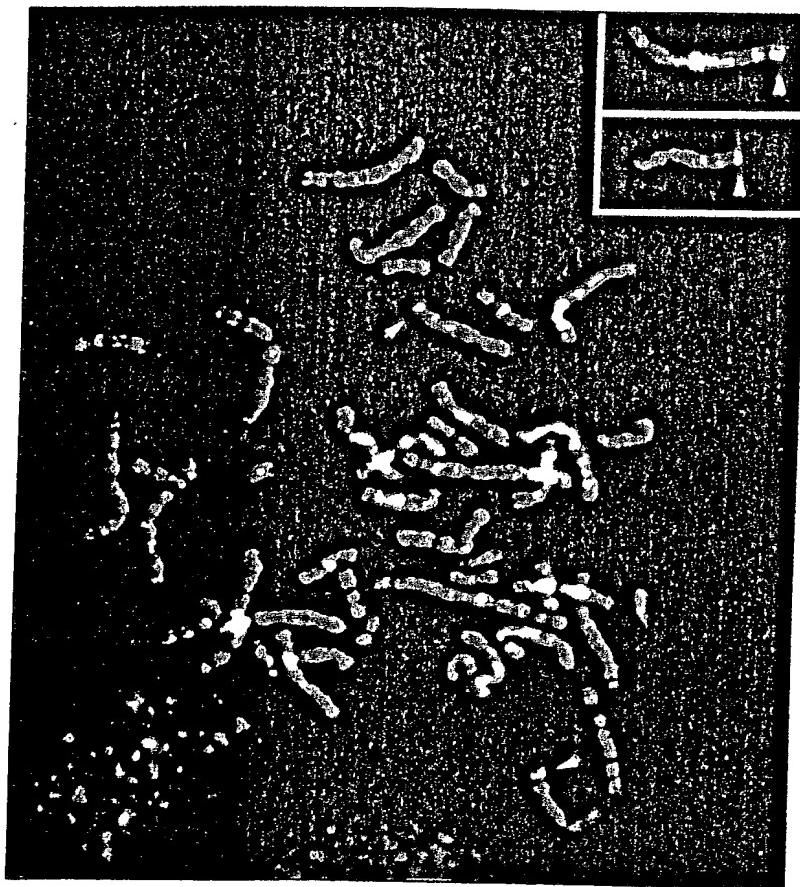


FIG. 14A

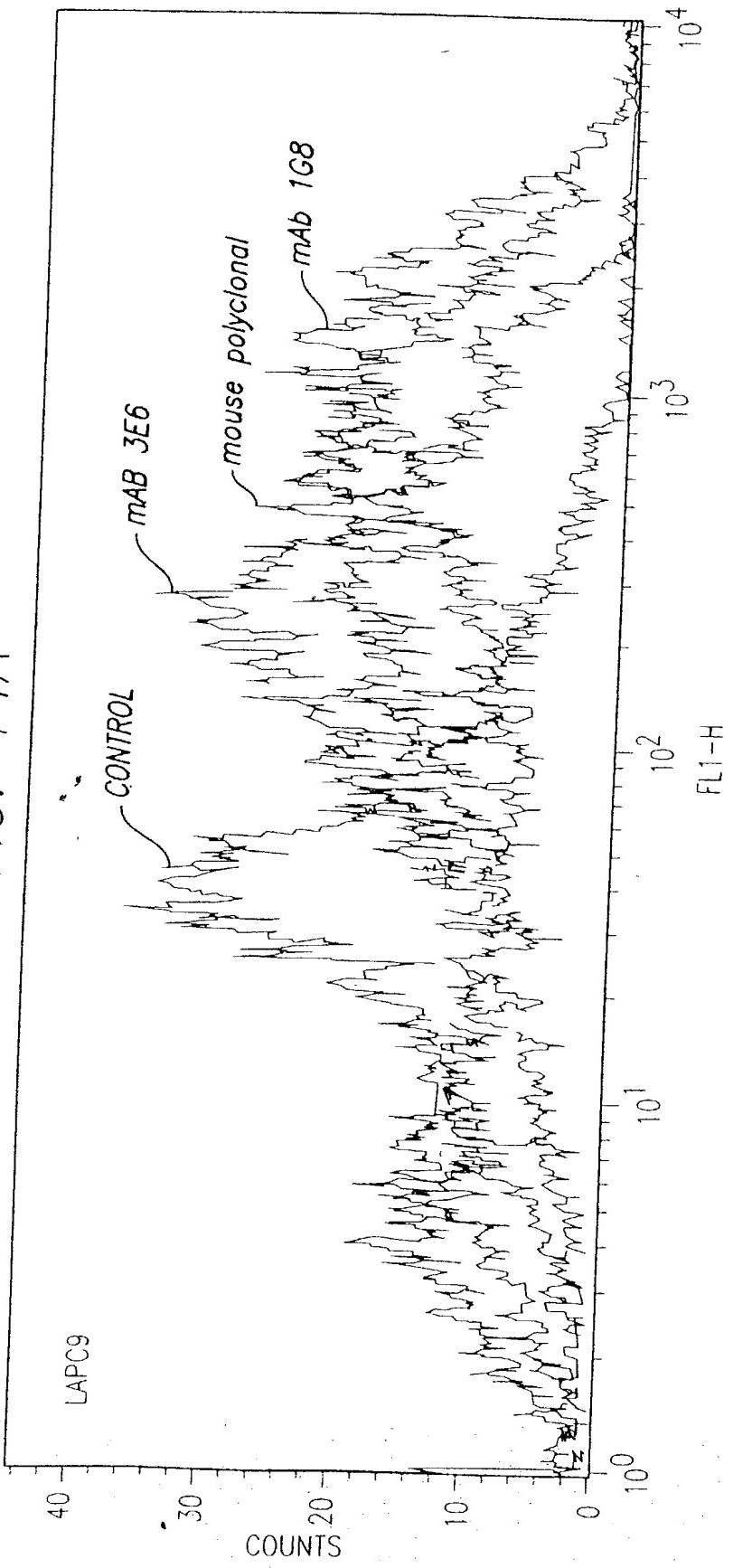


FIG. 14B

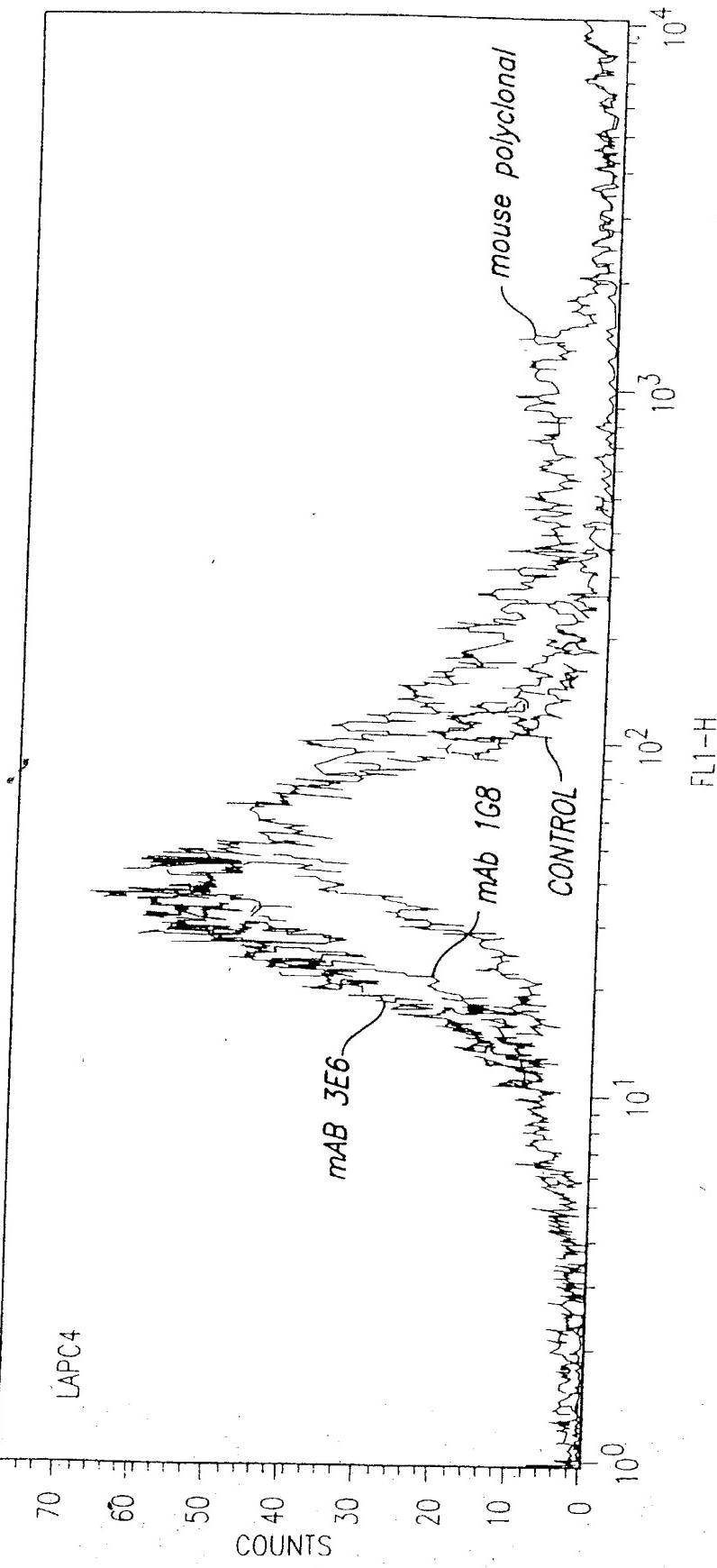
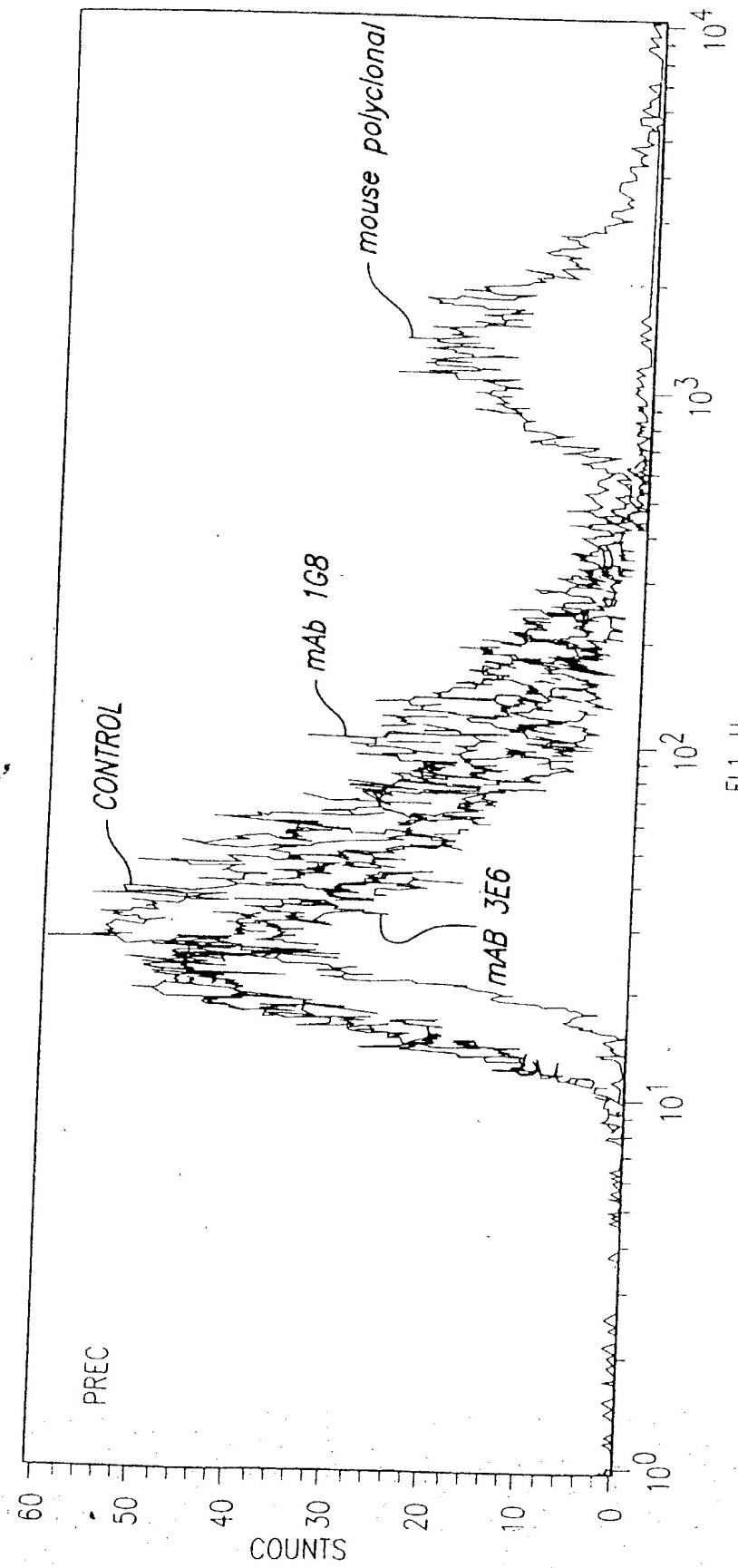


FIG. 14C



EPIIOPIC MAP

mAb	ISOIYPE	FL (18-98)	N (2-50)	M (46-109)	C (85-125)
1G8	IgG1	k	2.039	0.007	0.628
2H9	IgG1	k	1.318	0.863	0.032
3C5	IgG2a	k	2.893	1.965	0.016
3E6	IgG3	k	0.328	0.024	0.069
4A10	IgG2a	k	2.039	1.315	0.000
2A2	IgG2a	k	1.366	0.733	0.010
3G3	IgG2a	k	2.805	1.731	0.004

FIG. 15A

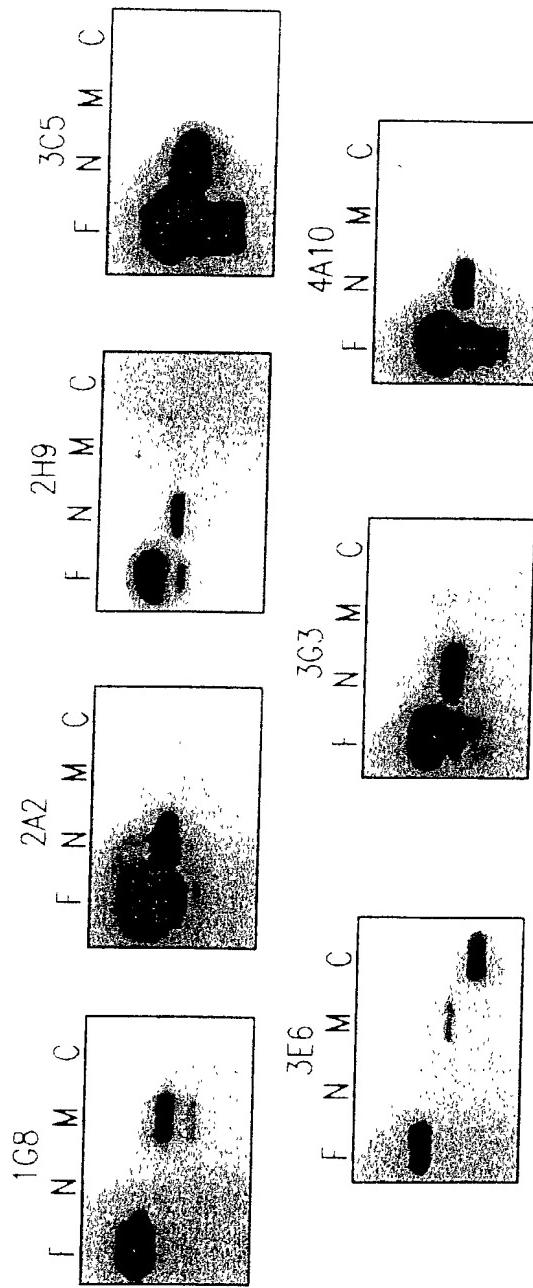


FIG. 15B

PROSTATE STEM CELL ANTIGEN (PSCA) IS A GPI-ANCHORED PROTEIN

1	M K I F L P V	T S A A L E G V E R A S S	hSCA-2
1	M K A Y E L A	I S M A C I T A E Q P G T A	hPSCA
1	M K T Y E F L	E L E T A T Y C I A L H P G A A	mPSCA
21	F M C F S C	L N Q K S I N L Y C I K P T I	
21	C L C Y S C	S C K A Q V S I N * D C E N Y Q N *	
21	C S L Q C	S C T A Q M N N * R D C E N Y Q N *	
41	G S D Q D N Y	G V T V S A S A G I G N L	
41	G T Q L G E Q	G W T A R I R A V G L L T	
41	G S L D Q H S	G F T S R I R A I G L L T	
61	V T F G H S L	K T C S P A C P I P E G	
61	- - - - -	S K G E S L N G V D D S Q	
61	- - - - -	S C F T S Q G E D D S E	
61	V N V G V A S M	G T S C Q S F E C N * F	
76	D Y Y V G K K	G T S C Q D T D C N A	
76	N Y Y L G K K	G T S C Q Y S D C N V	
81	S A A D G G L R A S V T	G T S C Q S F E C N * A	
101	S G A A H A	G T S C Q D T D C N A	
95	N G A A H T	G T S C Q Y S D C N V	
95	K E P T T L G F E L T V E C S	G T S C Q Y S D C N V	
121	S C E P A L L R F G P	G T S C Q Y S D C N V	
115	N C E P G Q -	G T S C Q Y S D C N V	
115	N C S S R -	G T S C Q Y S D C N V	

FIG. 16A

PROSTATE STEM CELL ANTIGEN (PSCA) IS A GPI-ANCHORED PROTEIN

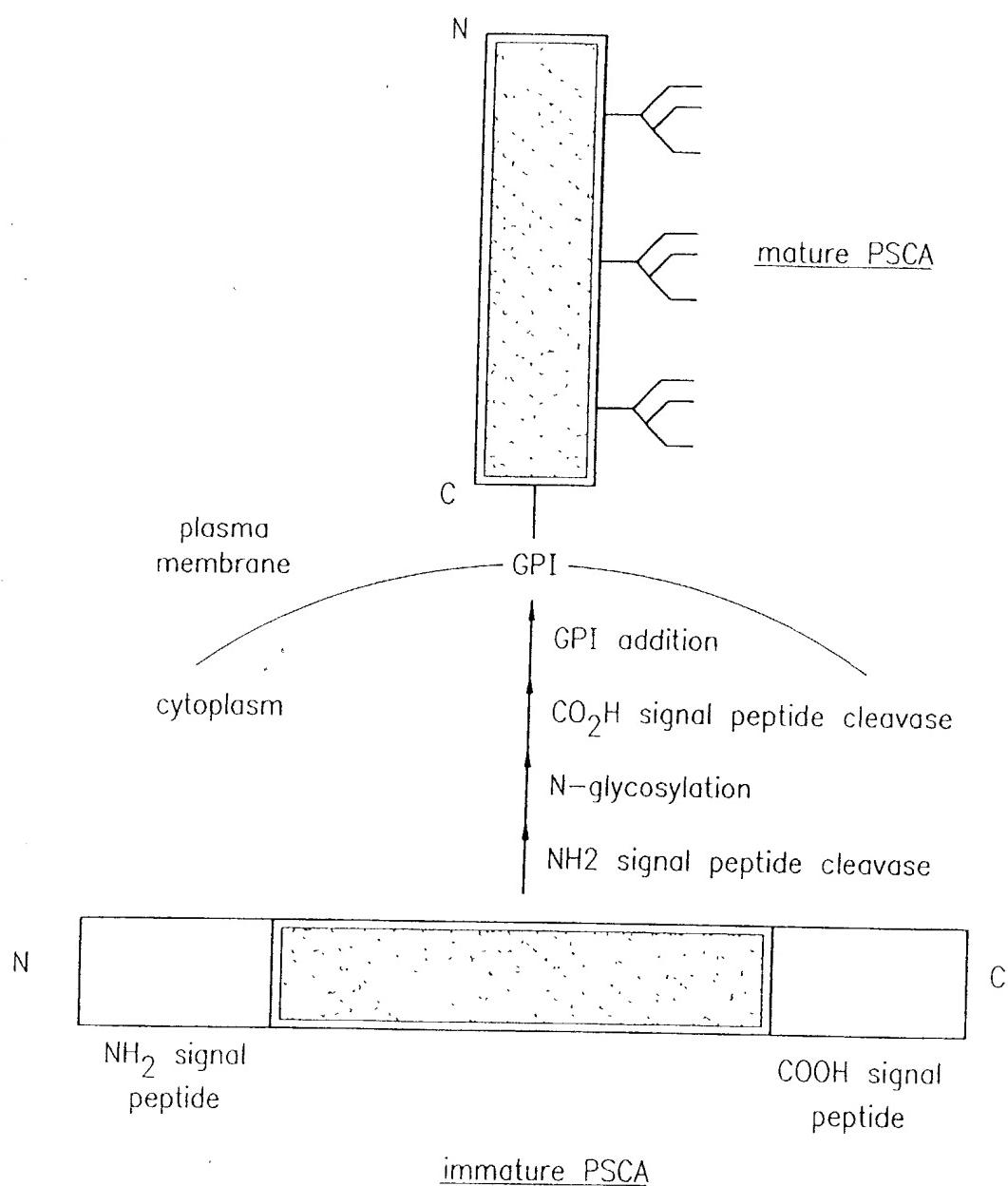
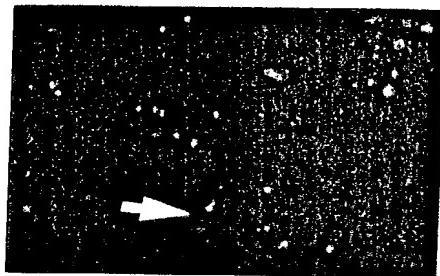


FIG. 16B

FIG. 17

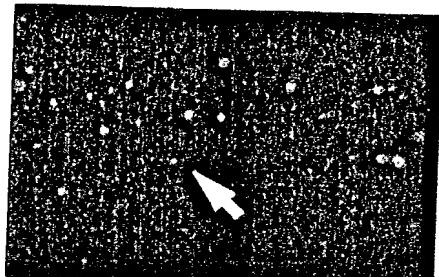
FISH ANALYSIS OF PSCA AND c-myc IN PROSTATE CANCER

GAIN CHROMOSOME 8



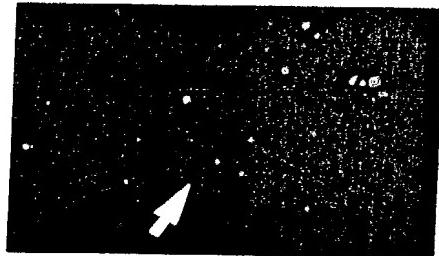
#34 c-myc

AMPLIFICATION



#75 c-myc

#34 PSCA



#75 PSCA

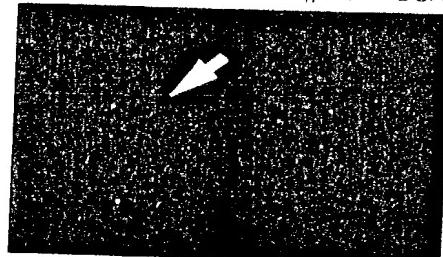
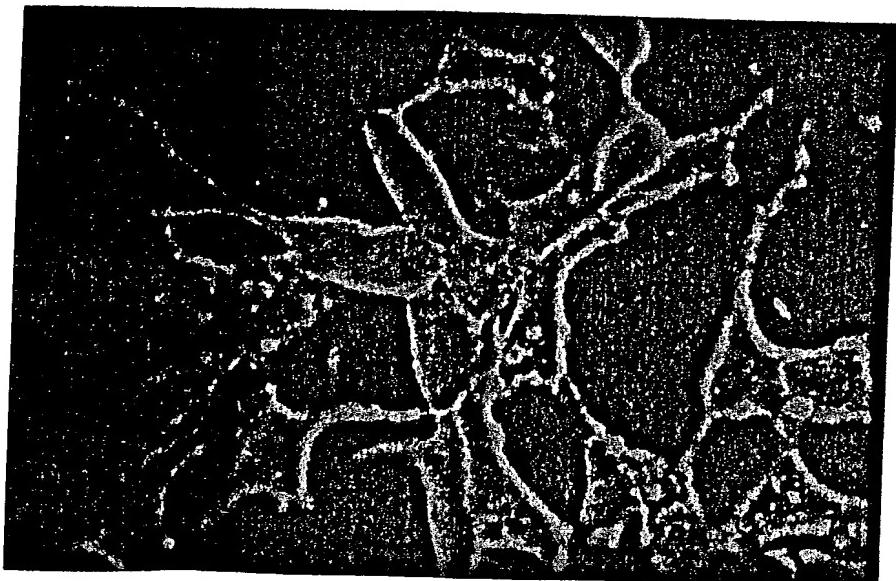


FIG. 18



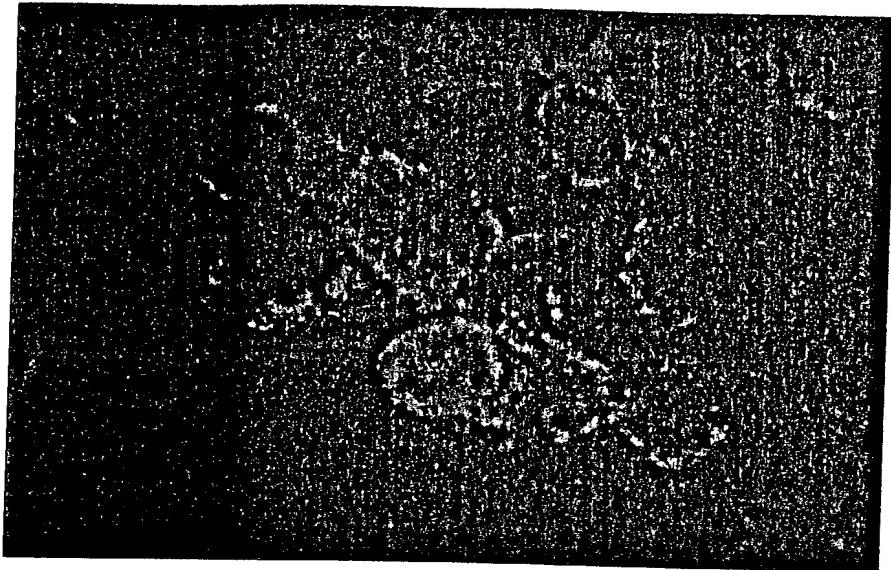


FIG. 19

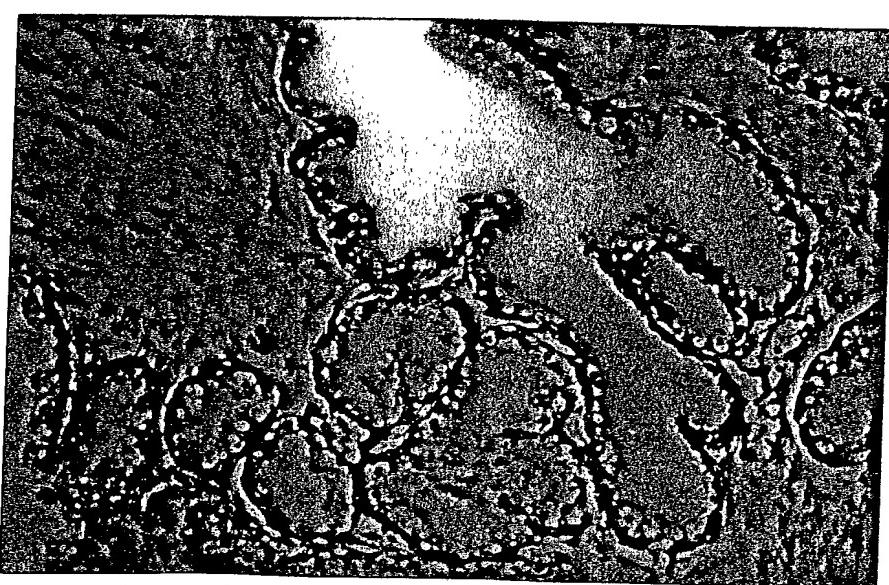


FIG. 20

FIG. 21

PSCA IMMUNOSTAINING OF PRIMARY TUMORS

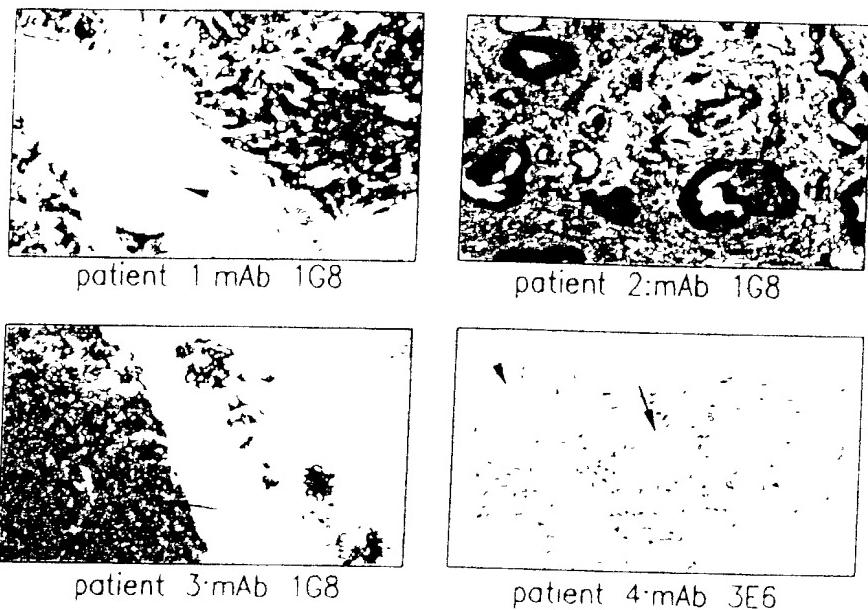


FIG. 22

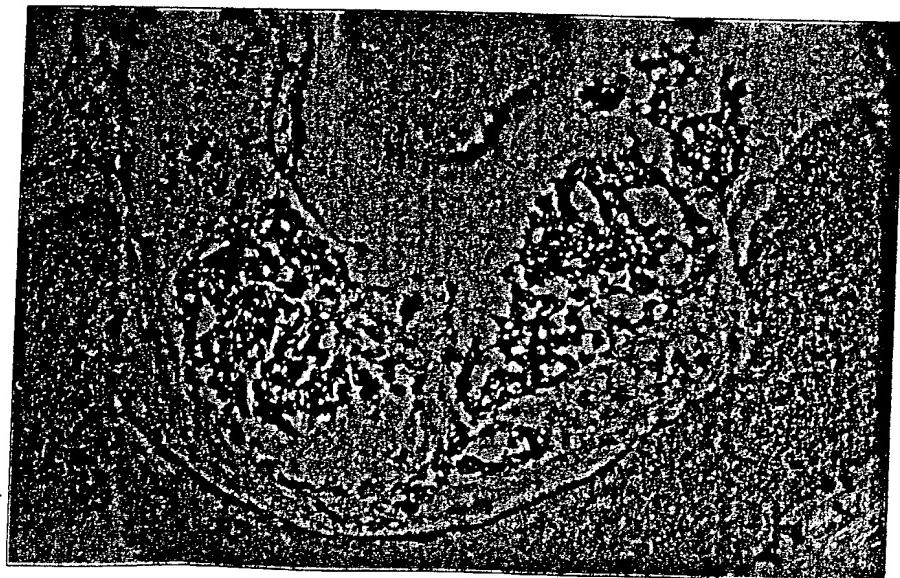




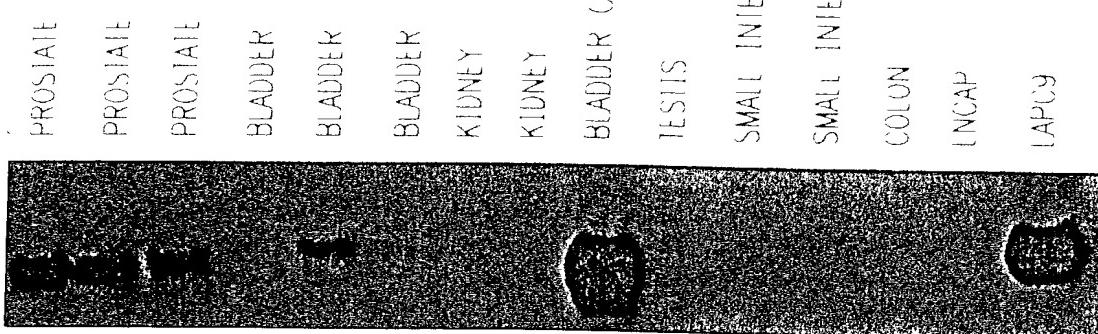
FIG. 23

FIG. 24



PROSTATE PROSTATE PROSTATE PROSTATE

FIG. 25



DSCA NORTHERN

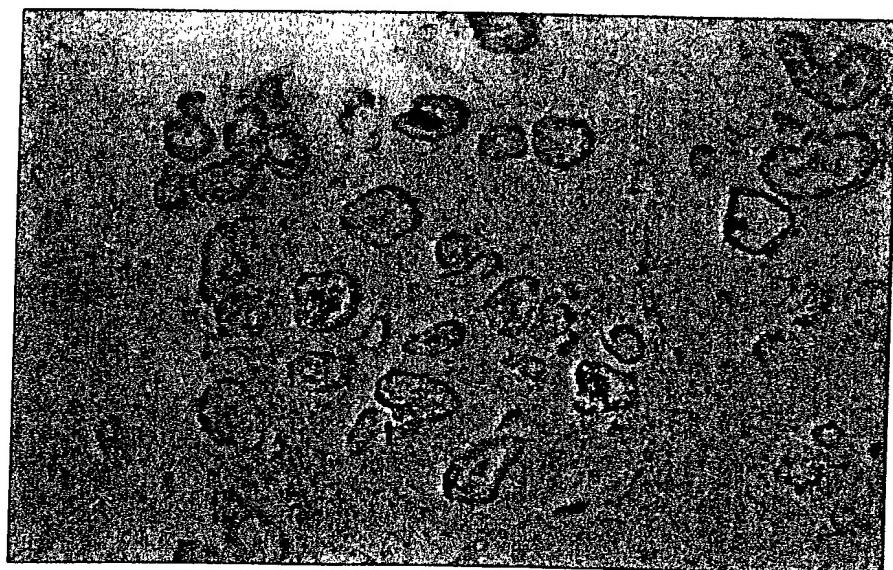


FIG. 26

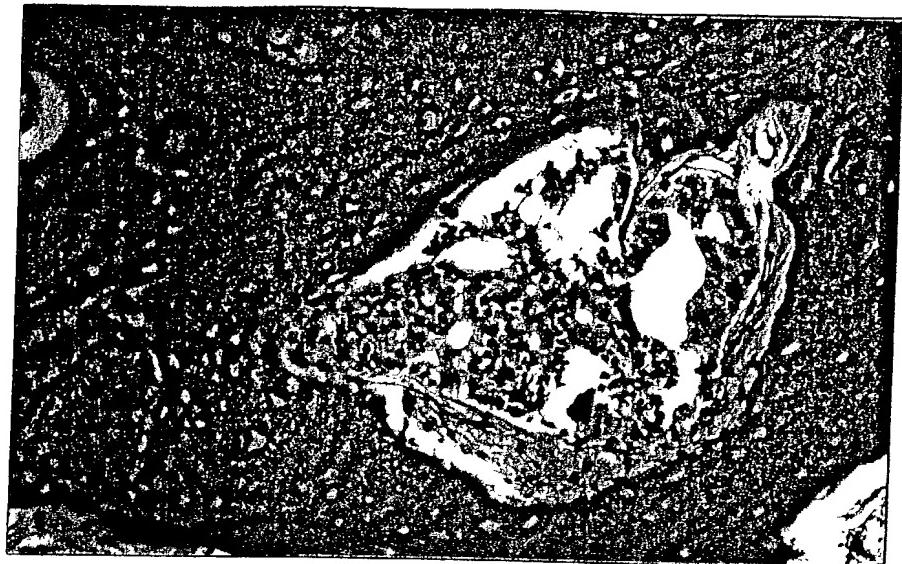
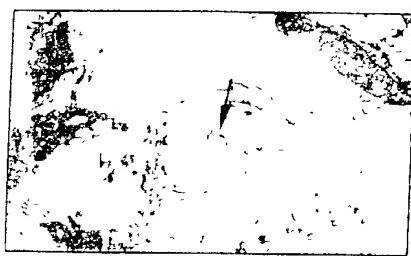


FIG. 27

PSCA IMMUNOSTAINING OF BONY METASTASES



Patient 5: H and E
and mAb 1G8



Patient 4: H and E
and mAb 3E6

FIG. 28

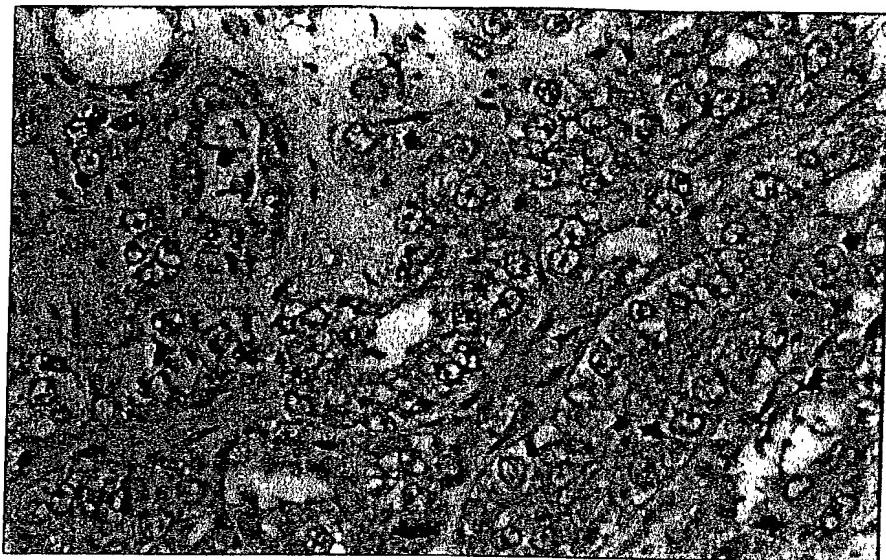
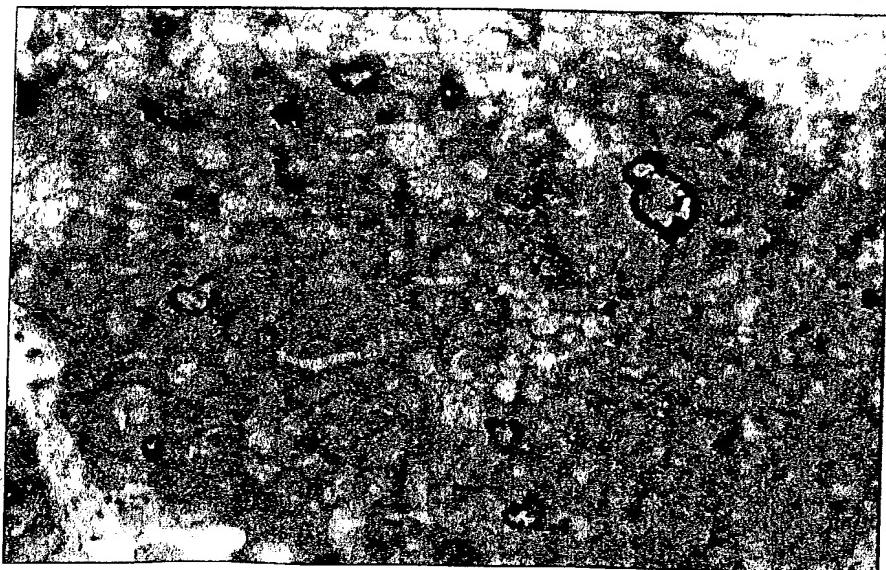


FIG. 29

FIG. 30



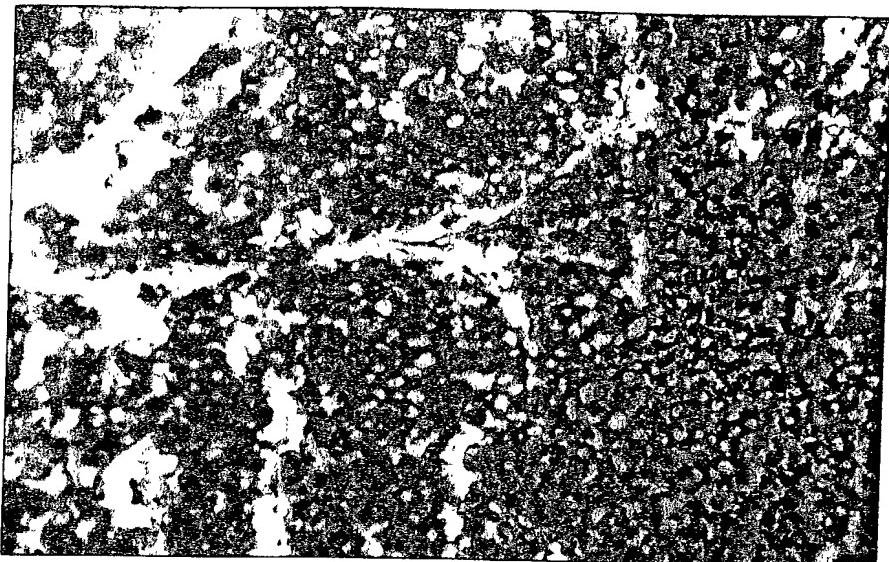


FIG. 31

FIG. 32

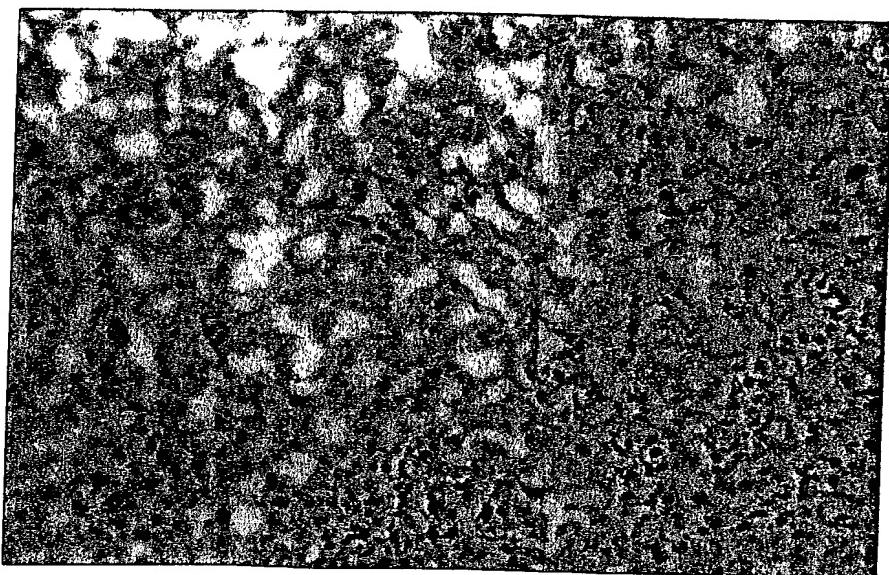


FIG. 33

PSCA EXPRESSION IN LAPC-9 XENOGRAFT BY FACS

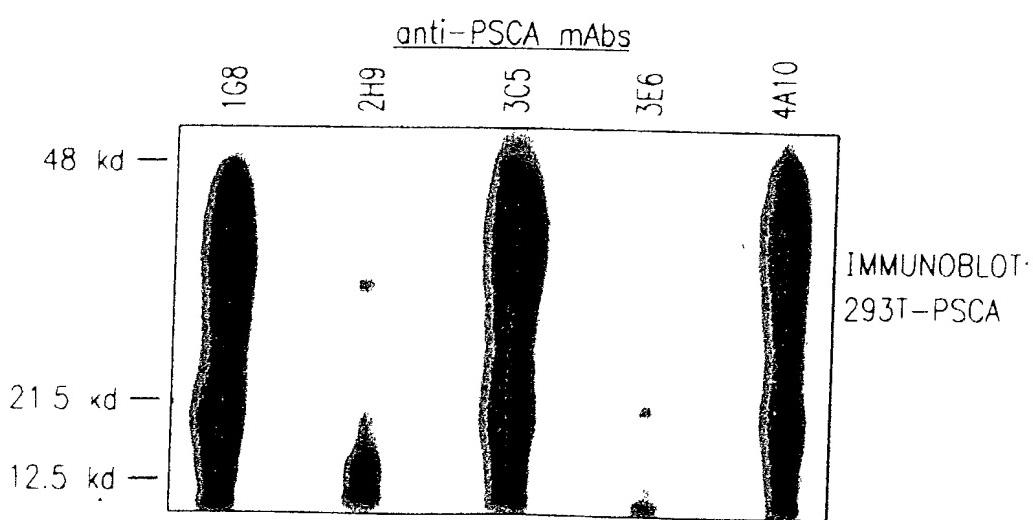
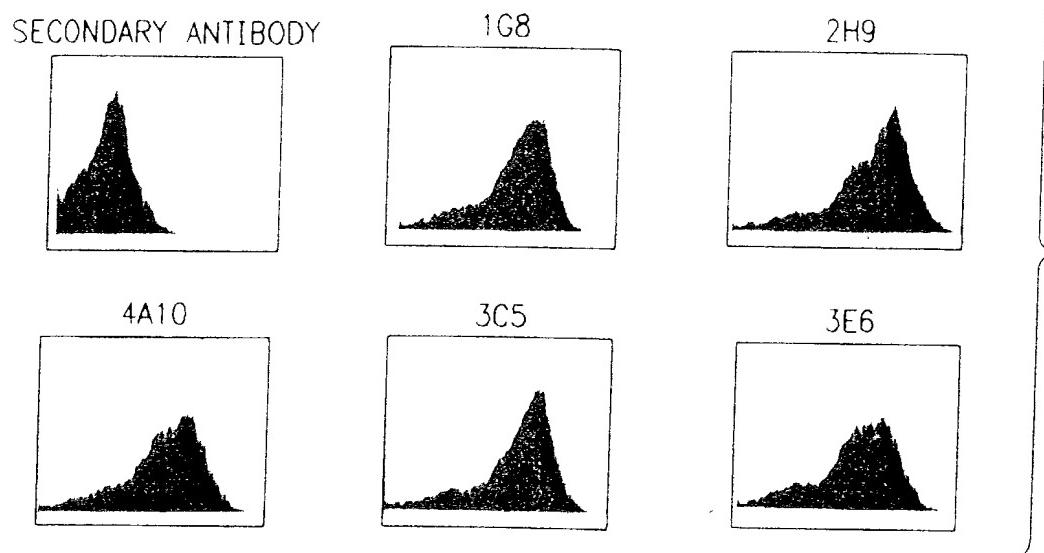


FIG. 34

FIG. 35

IMMUNOFLUORESCENT STAINING OF LNCaP-PSCA CELLS

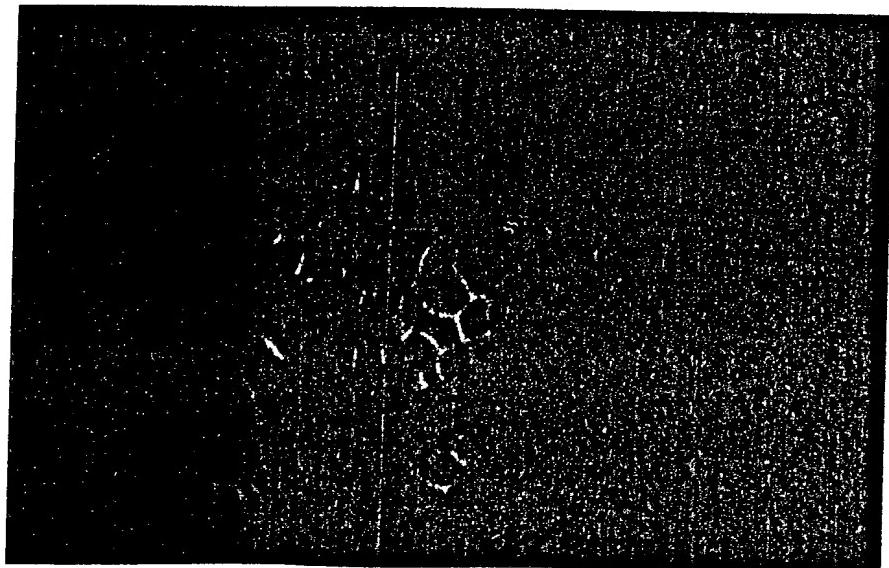
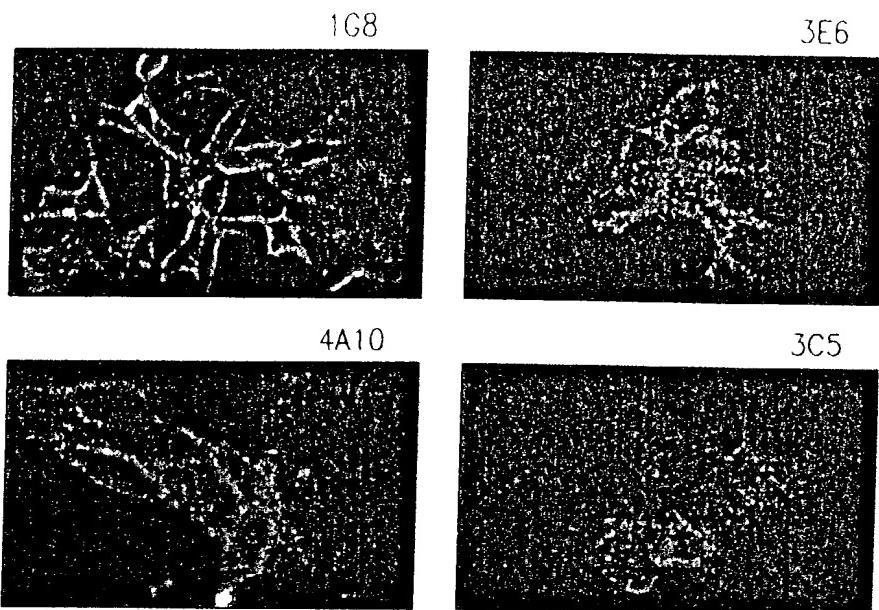


FIG. 36

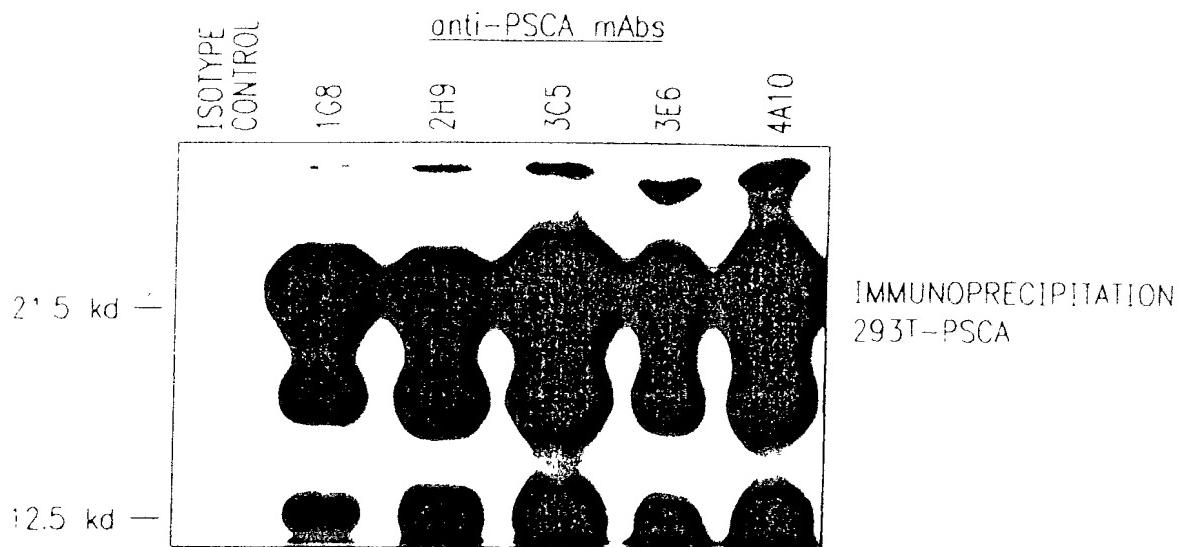


FIG. 37

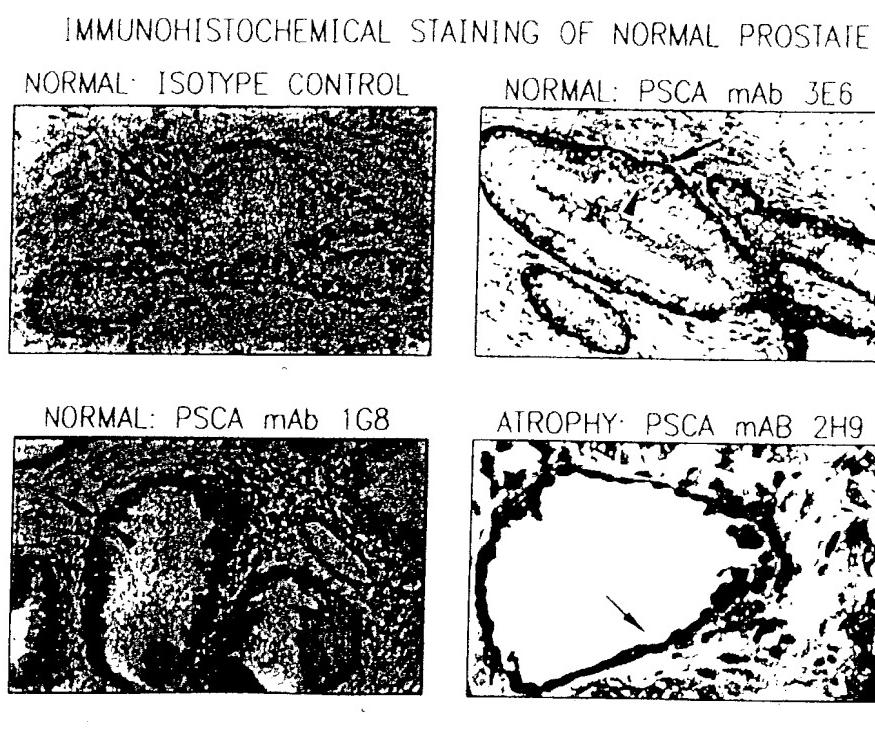


FIG. 38

FIG. 39A

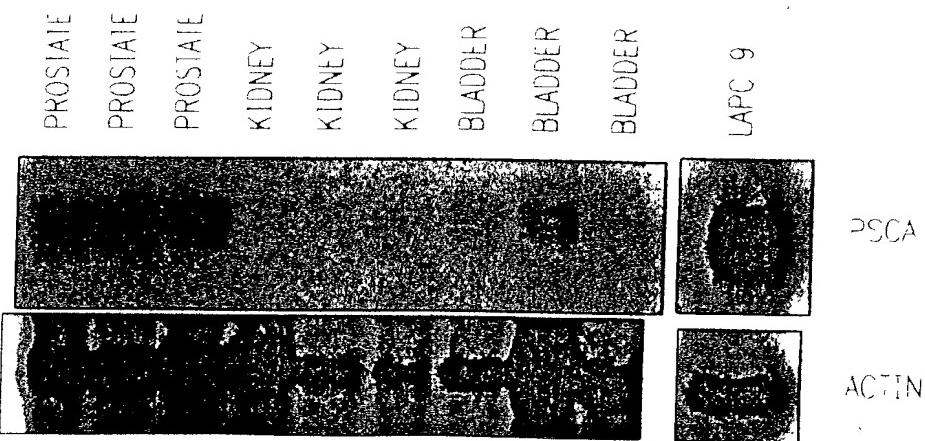
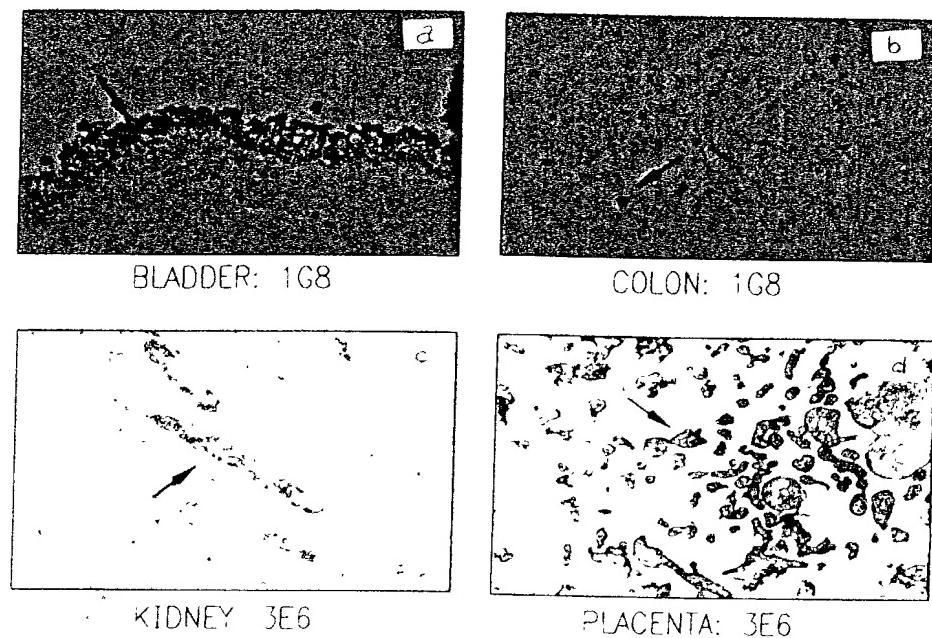
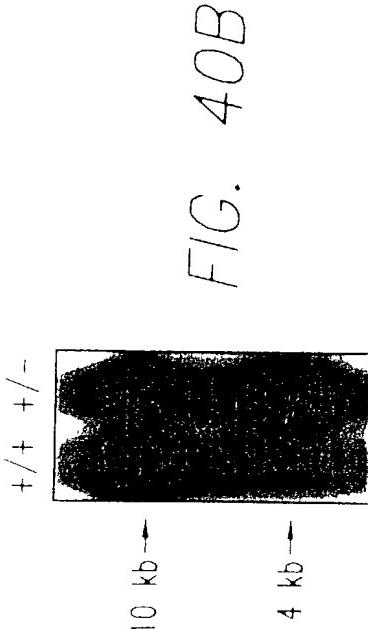
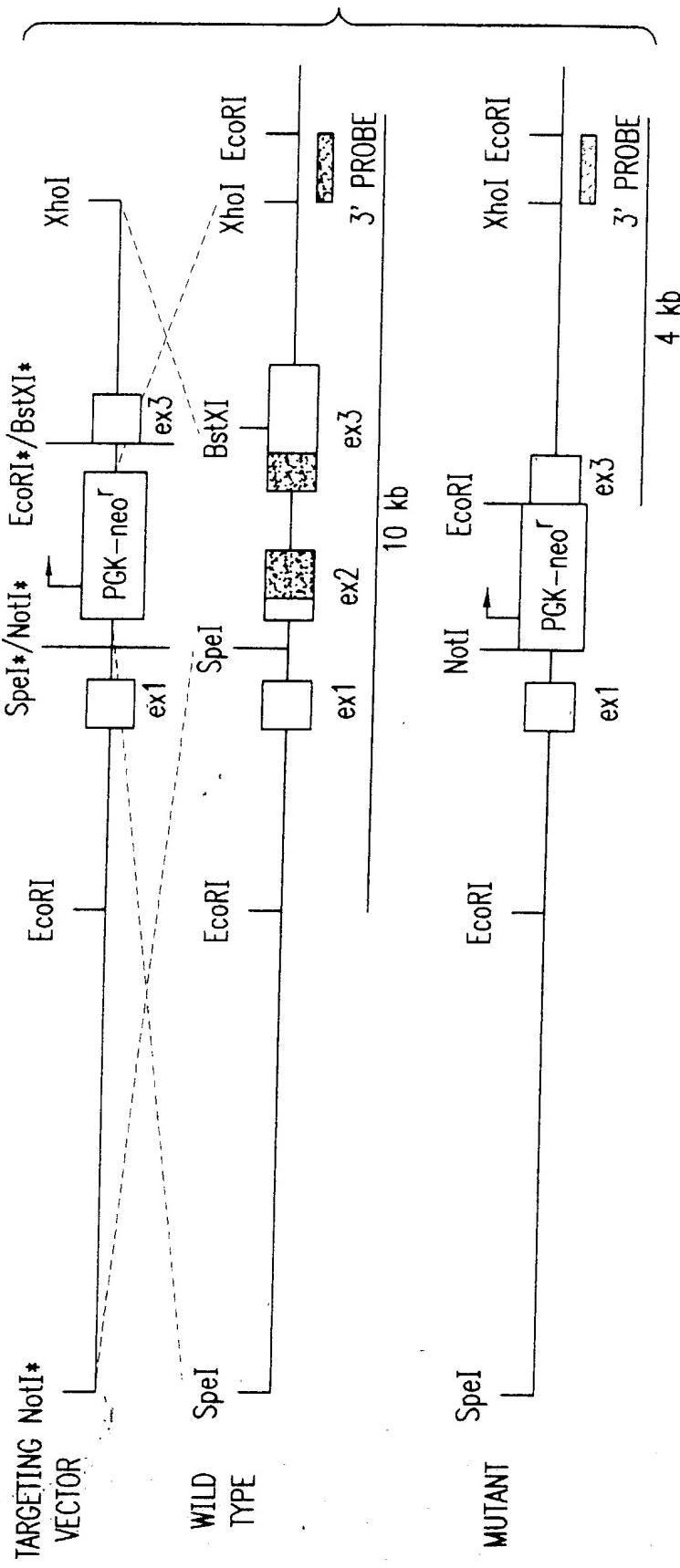


FIG. 39B

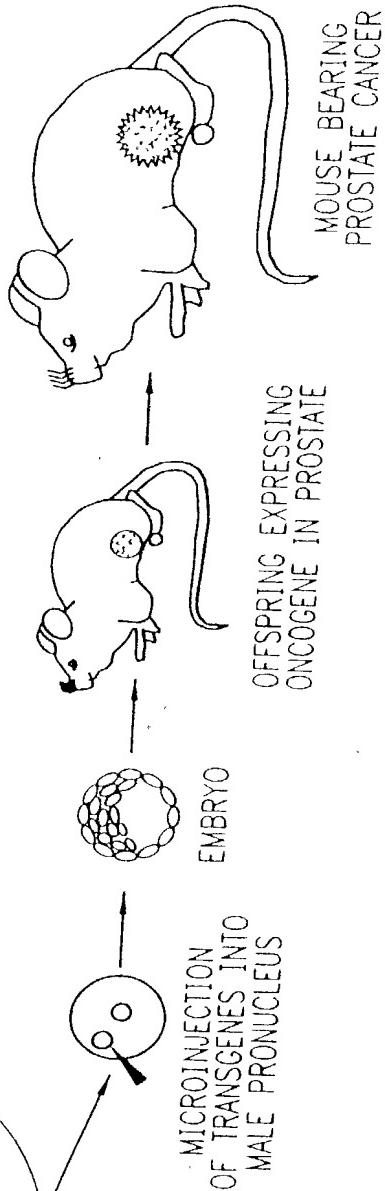
FIG. 40A



PROSTATE
SPECIFIC
PROMOTER

TRANSGENIC MOUSE MODELS OF PROSTATE CANCER

FIG. 41



TRANSGENE	TARGET TISSUES	CHARACTERISTICS
C3(1) (-3 kb)/ SV40 LARGE+SMALL, T MAROULAKOU et al. 1994 PNAS	PROSTATE (SECRETORY CELLS) URETHRAL, MAMMARY AND SWEAT GLAND	LOW-GRADE PIN 8-12 WKS HIGH-GRADE PIN 8-12 WKS INVASIVE CARCINOMA 28 WKS NO METASTASES
PROBASIN (-426 bp)/ SV40 LARGE+SMALL, T GREENBERG et al. 1995 PNAS	PROSTATE (SECRETORY CELLS)	LOW-GRADE PIN 5-8 WKS HIGH-GRADE PIN 8-12 WKS INVASIVE CARCINOMA 12 WKS METASTASES IN LYMPH NODE, LUNG, LIVER AND BONE
CRYPTDIN2 (-6.5 kb)/ SV40 LARGE+SMALL, T GARABEDIAN et al. 1998 PNAS	PROSTATE (NEUROENDOCRINE CELLS) SMALL INTESTINE	LOW-GRADE PIN 8-12 WKS HIGH-GRADE PIN 8-12 WKS INVASIVE CARCINOMA 16 WKS METASTASES IN LYMPH NODE, LUNG, LIVER, AND BONE

REPORTER GENE CONSTRUCTS FOR TRANSFECTION ASSAY

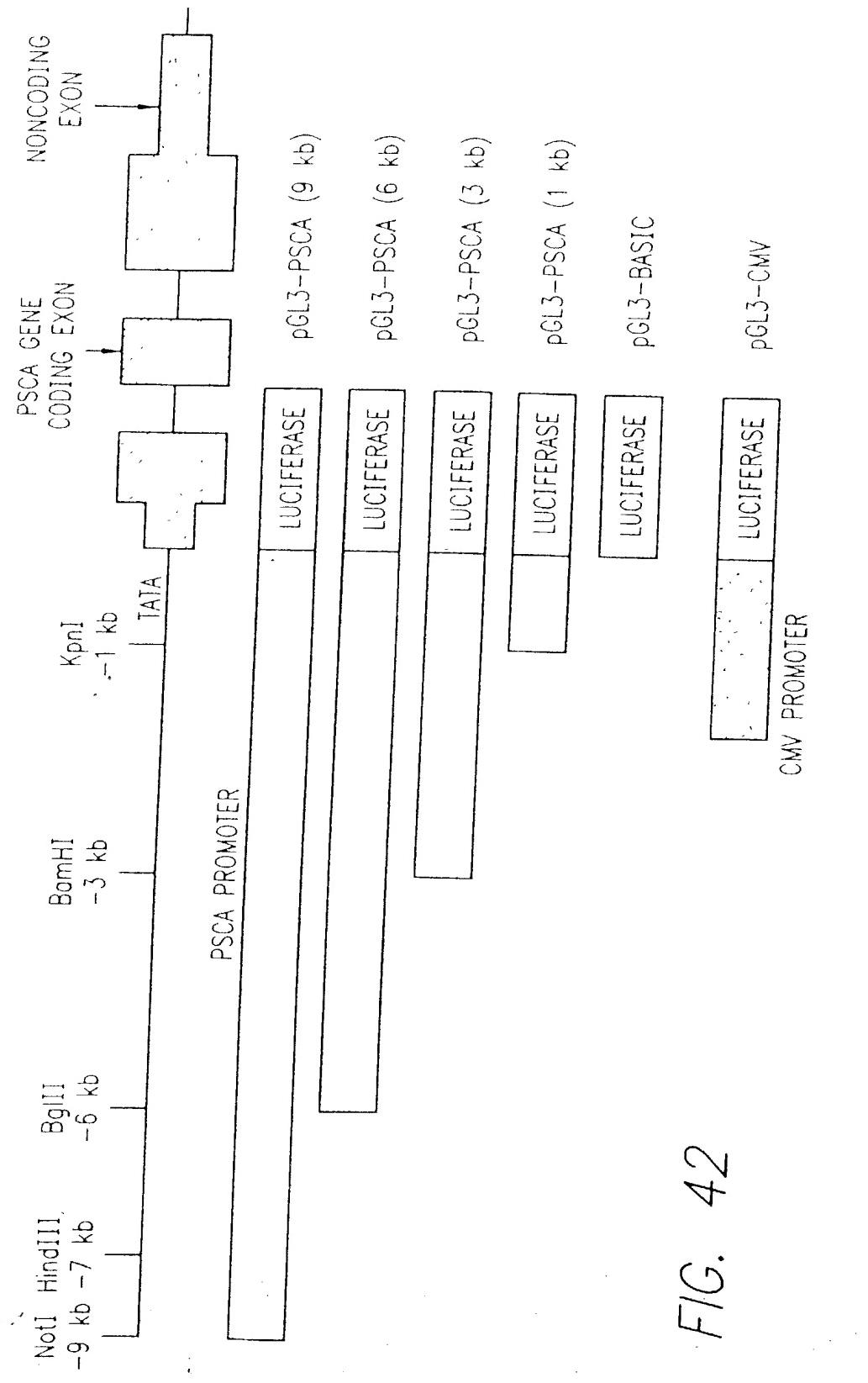


FIG. 42

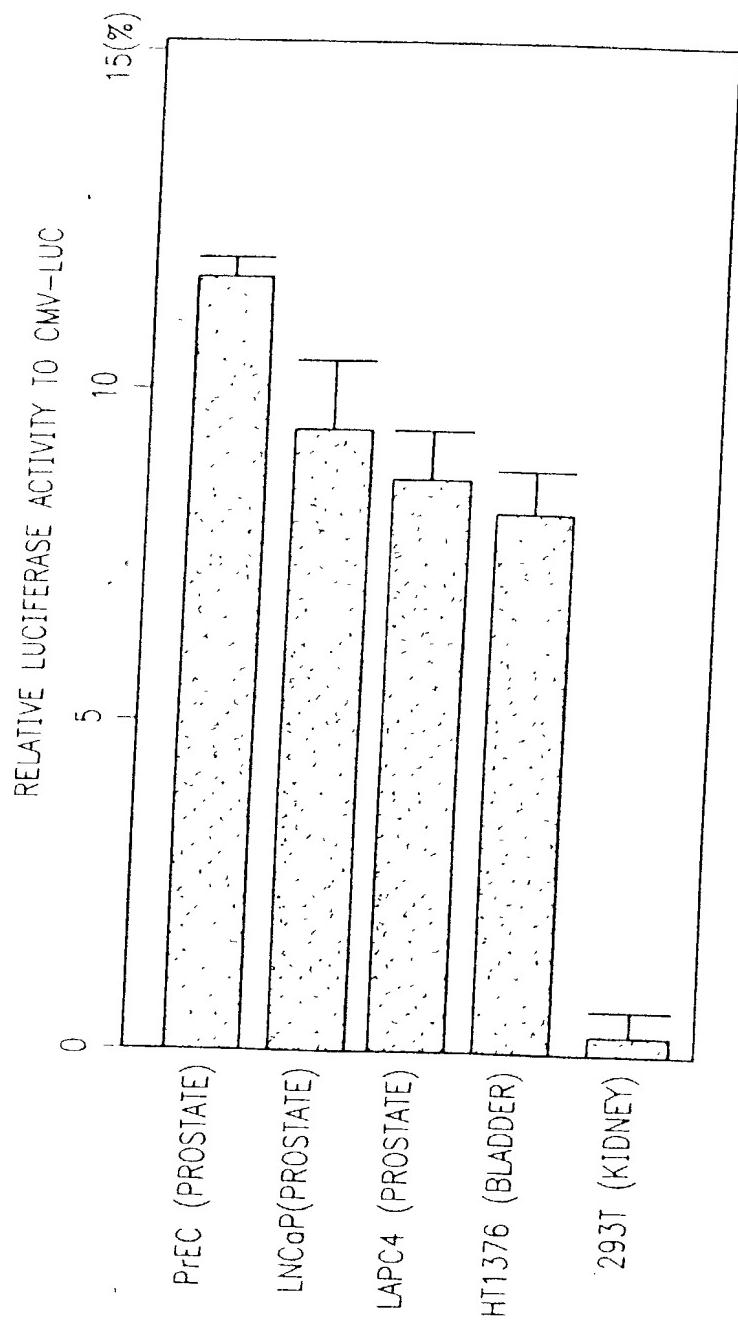


FIG. 4J

IDENTIFICATION OF PROSTATE-SPECIFIC ELEMENTS
WITHIN PSCA PROMOTER SEQUENCES

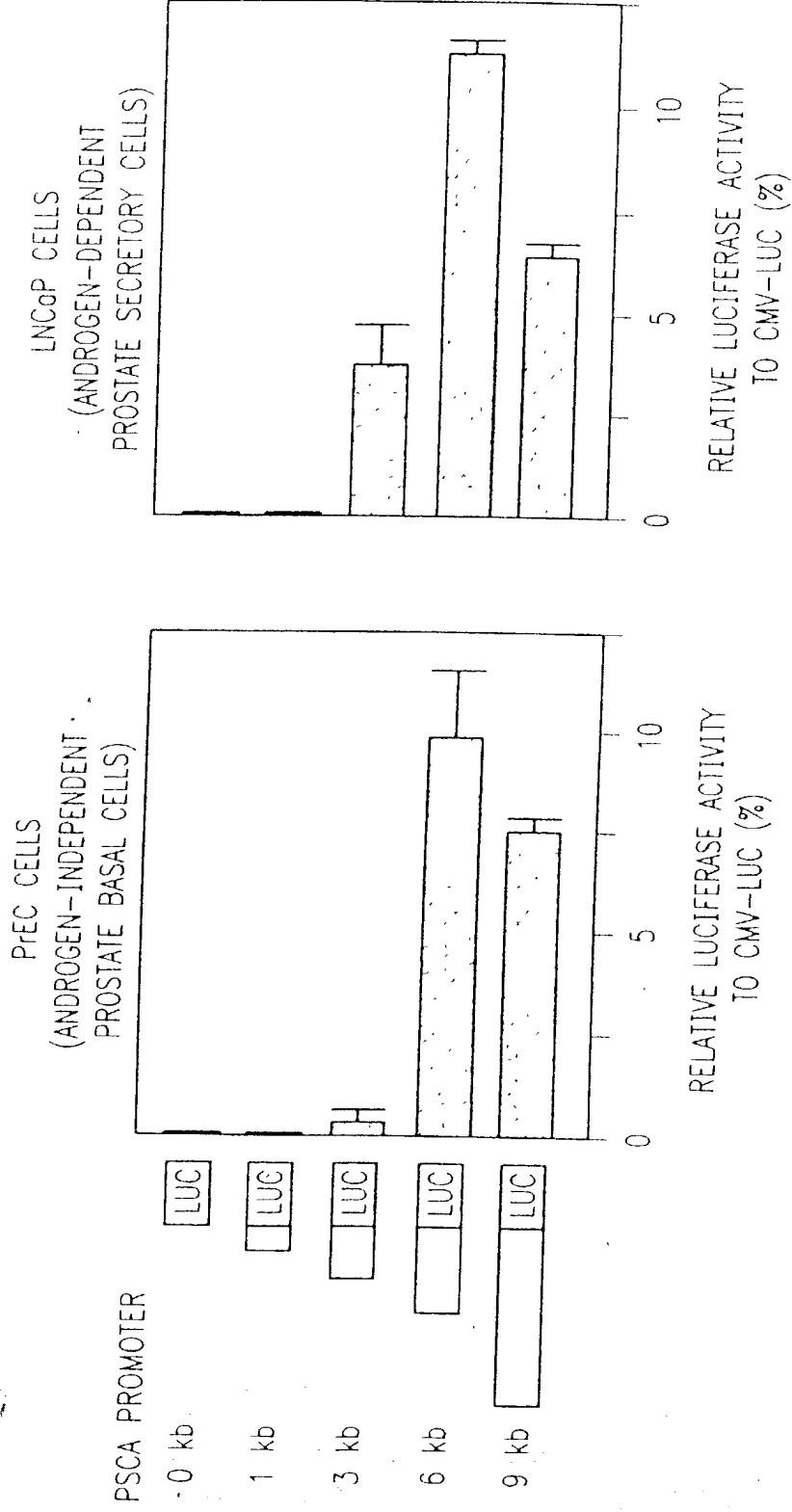
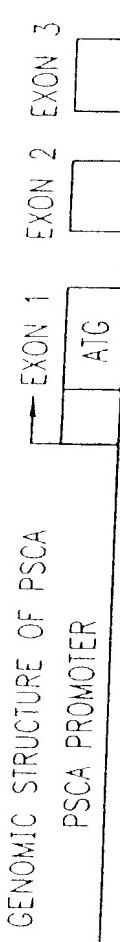


FIG. 44

FIG. 45

UPDATE OF TRANSGENIC MOUSE PROJECTS



	NUMBER OF FOUNDERS (DNA POSITIVE)
PSCA(9 kb)-GFP	2
PSCA PROMOTER (9kb)	1
PSCA(6 kb)-GFP	6
PSCA PROMOTER (6kb)	8
PSCA(9 kb)-GFP-3'hGH	3
PSCA PROMOTER (9kb)	9
PSCA(6 kb)-SV40TAG	3
PSCA PROMOTER (9kb)	3
PSCA(6 kb)-SV40TAG	9

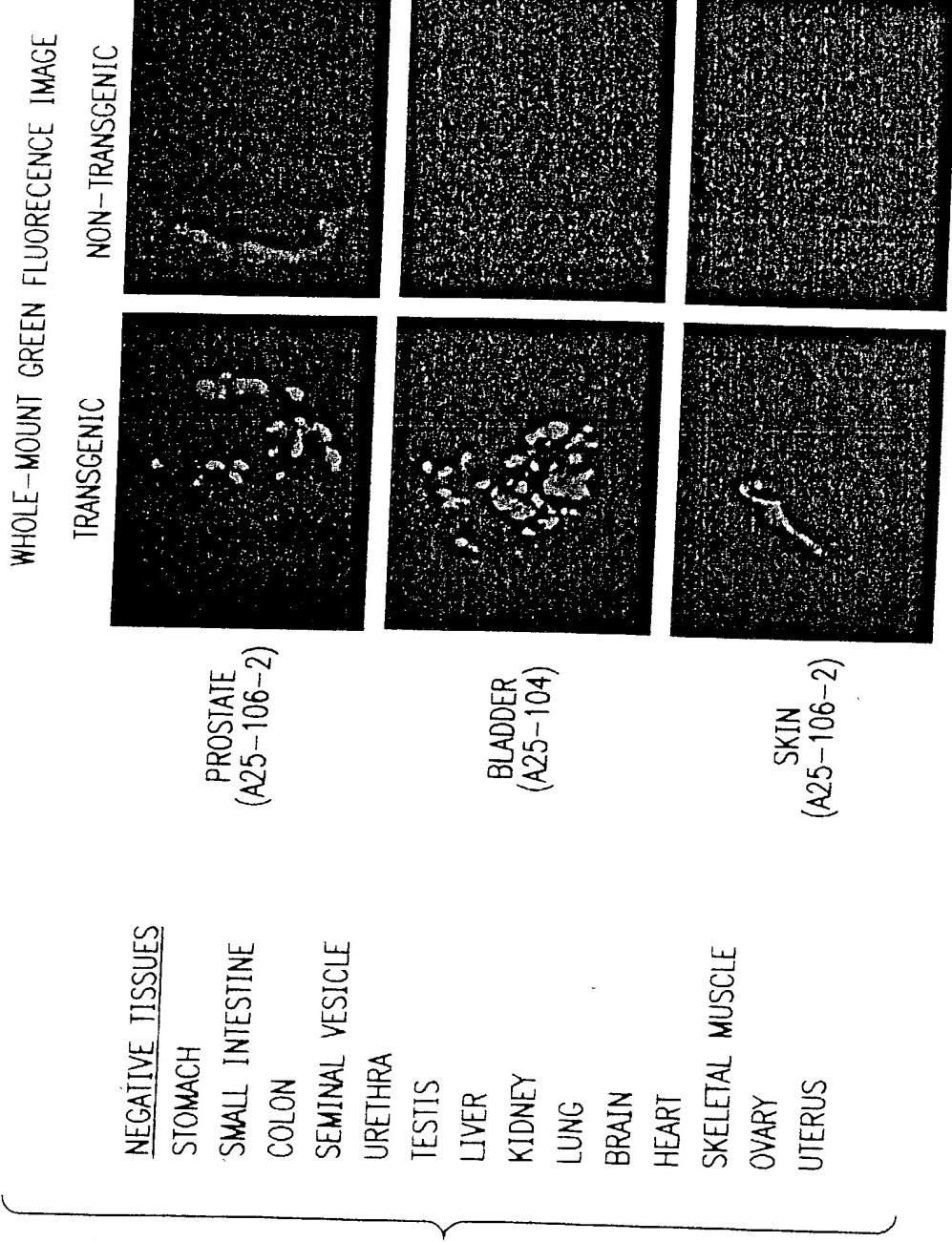


FIG. 46

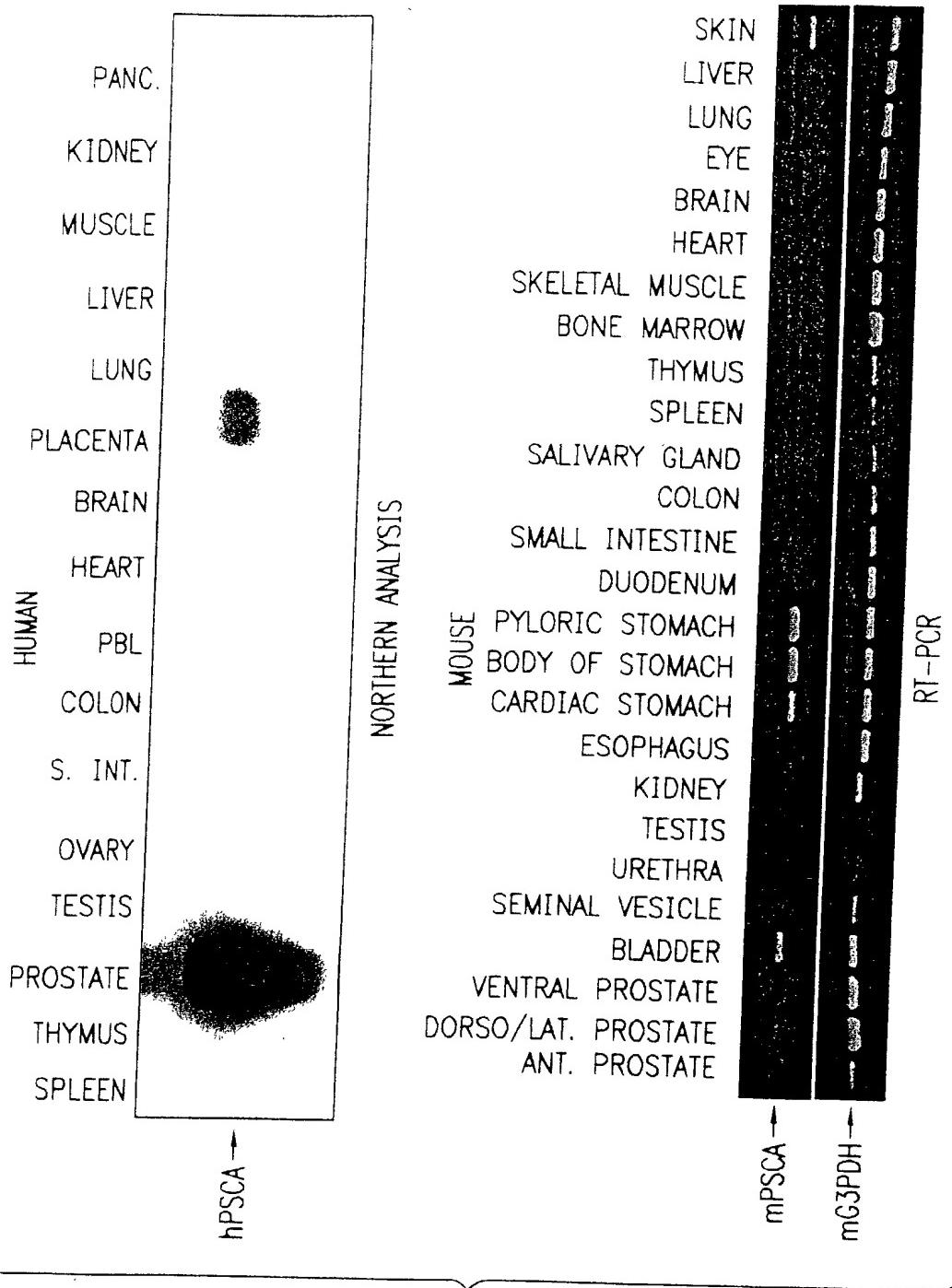
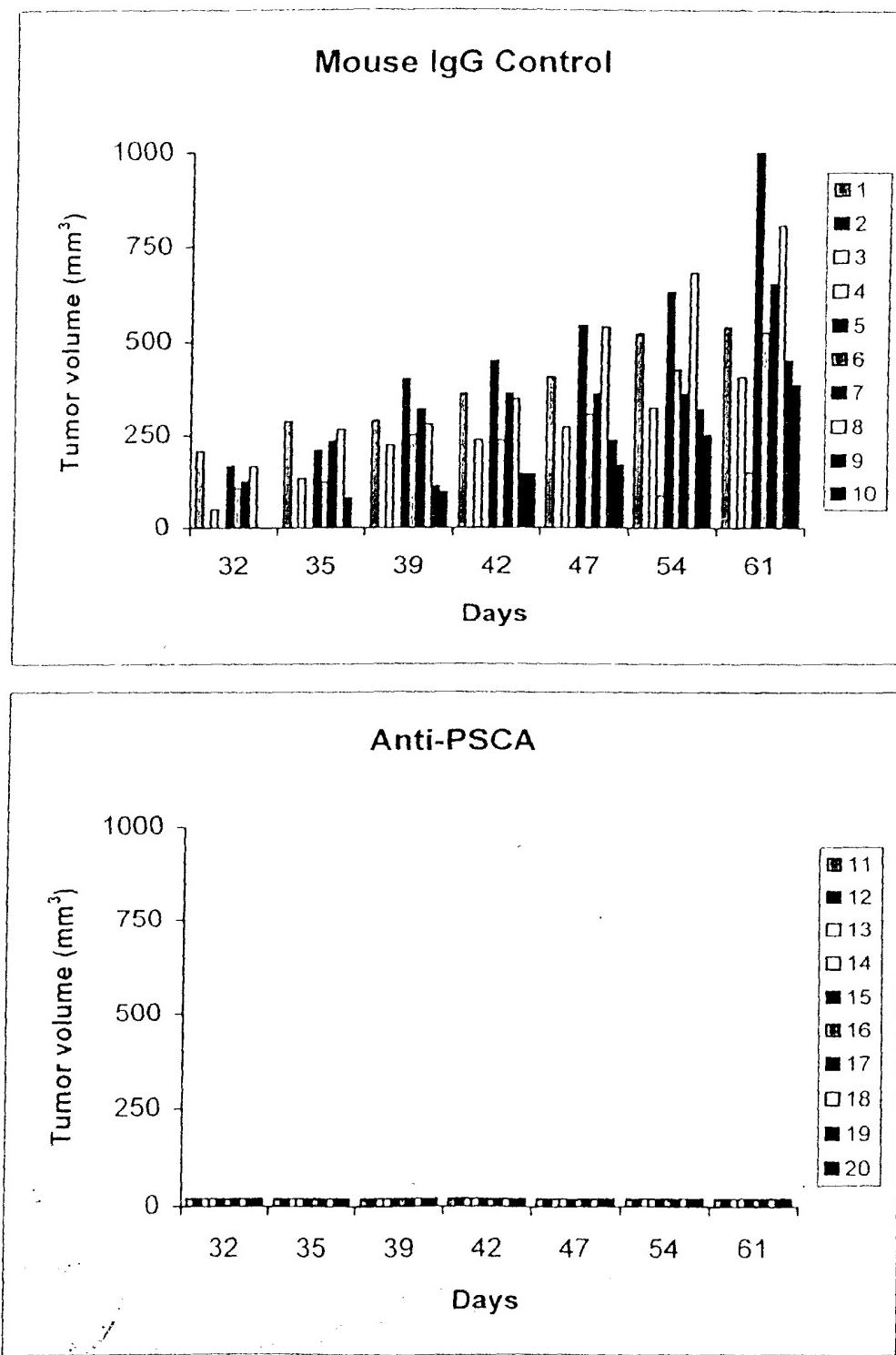


FIG. 47

FIG. 48



AEpitope recognized (OD 450 nm)

<u>mAb</u>	<u>F(18-98)</u>	<u>N(2-50)</u>	<u>M(46-109)</u>	<u>C(85-123)</u>
1G8	1.485	0.004	1.273	0.003
2A2	0.973	0.631	0.023	0.010
2H9	1.069	1.026	0.002	0.001
3C5	1.916	1.709	0.006	0.002
3E6	1.609	0.036	1.133	2.118
3G3	2.805	1.731	0.004	0.000
4A10	1.053	0.493	0.000	0.001

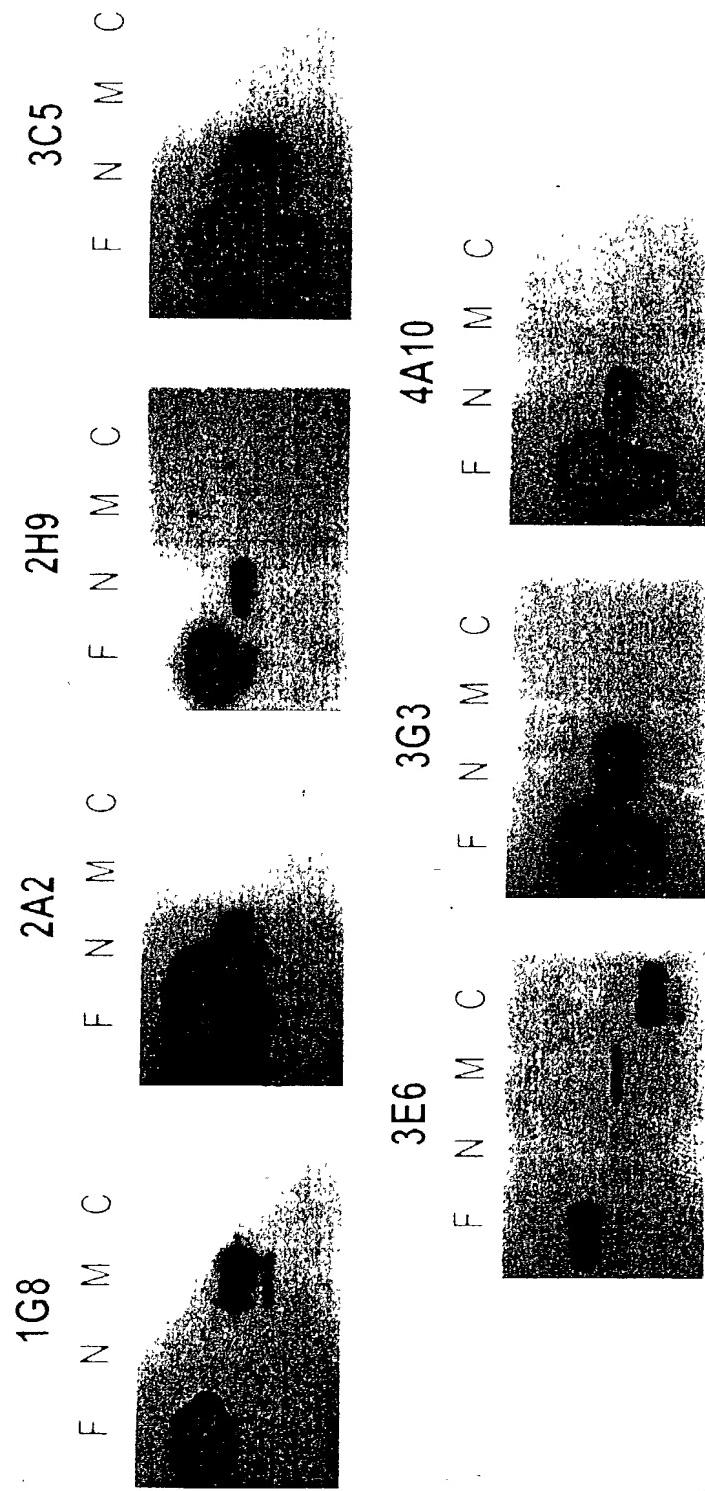
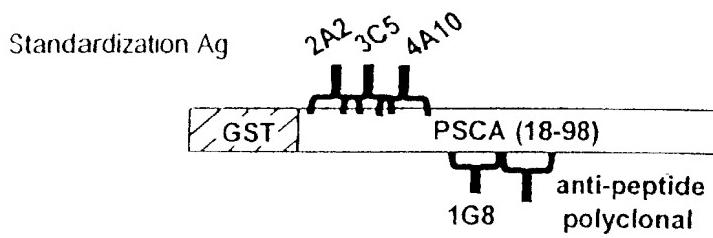
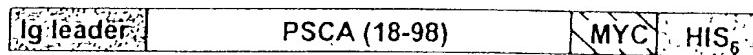
B

FIG. 50

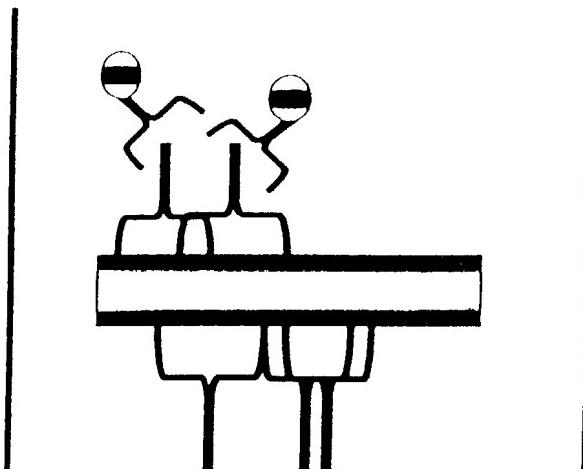
A



Engineered mammalian secreted form



B



Anti-IgG2a HRP

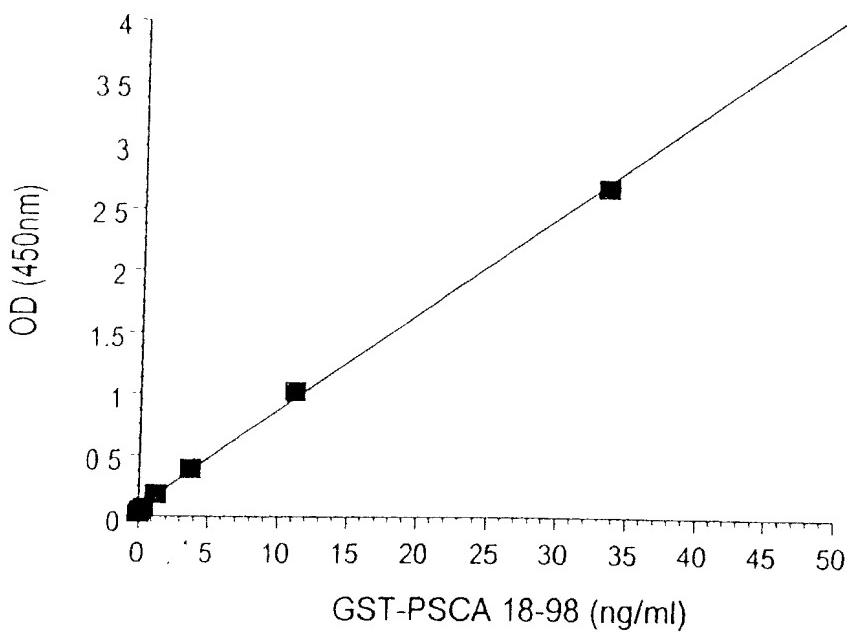
Anti-PSCA mAbs 3C5+4A10+2A2 (IgG2a)

PSCA

Affinity purified anti-peptide polyclonal
+ mAb 1G8 (IgG1)

FIG. 51

A



B

<u>Sample</u>	<u>OD+range (n=2)</u>	<u>ng/ml</u>
vector	0.005+0.001	ND
vector+hu serum	0.004+0.001	ND
secPSCA	2.695+0.031	32.92
secPSCA+hu serum	2.187+0.029	26.55

10 13 13 15 15 15 15 15 15 15 15 15 15 15

FIG. 52

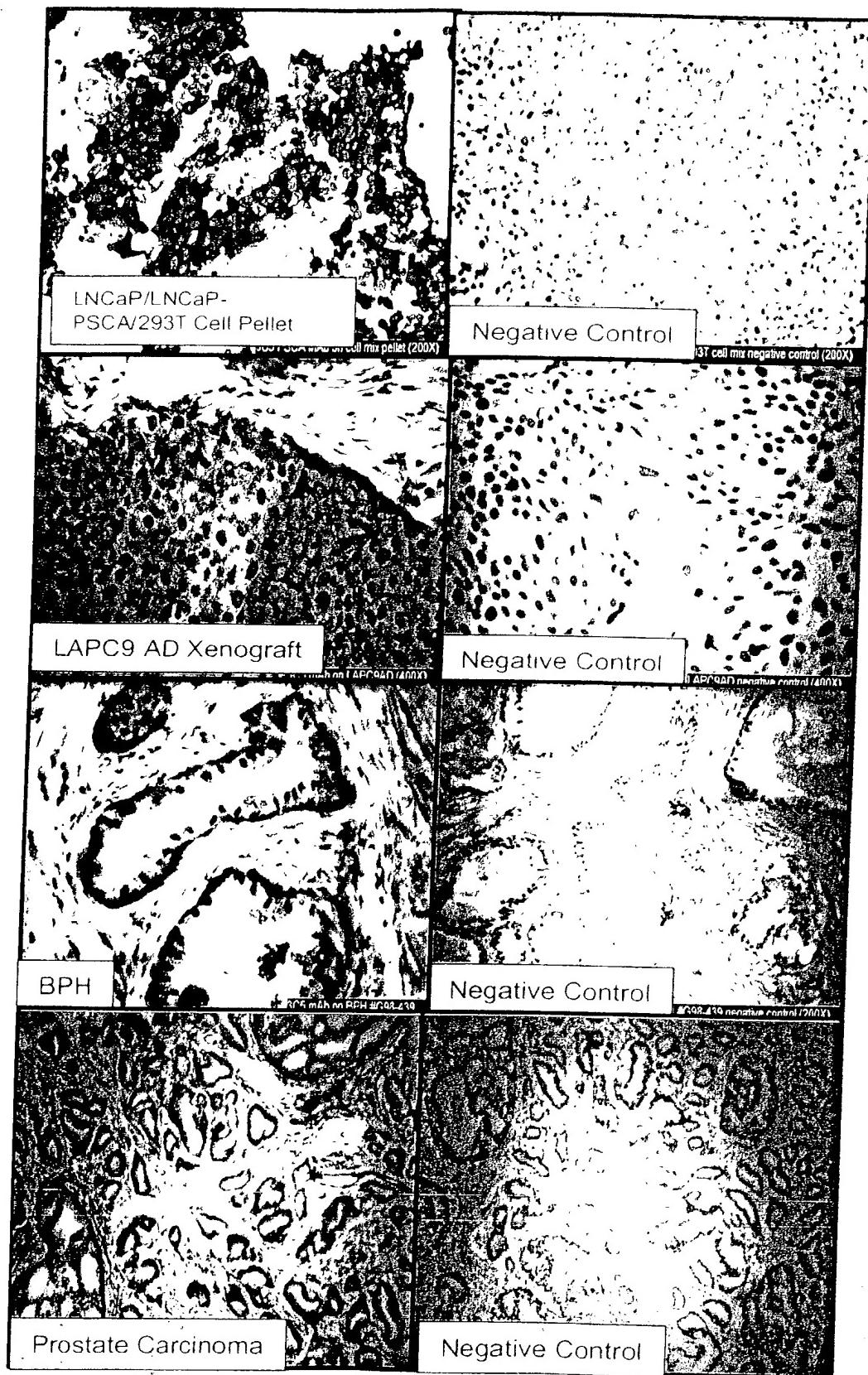


FIG. 53

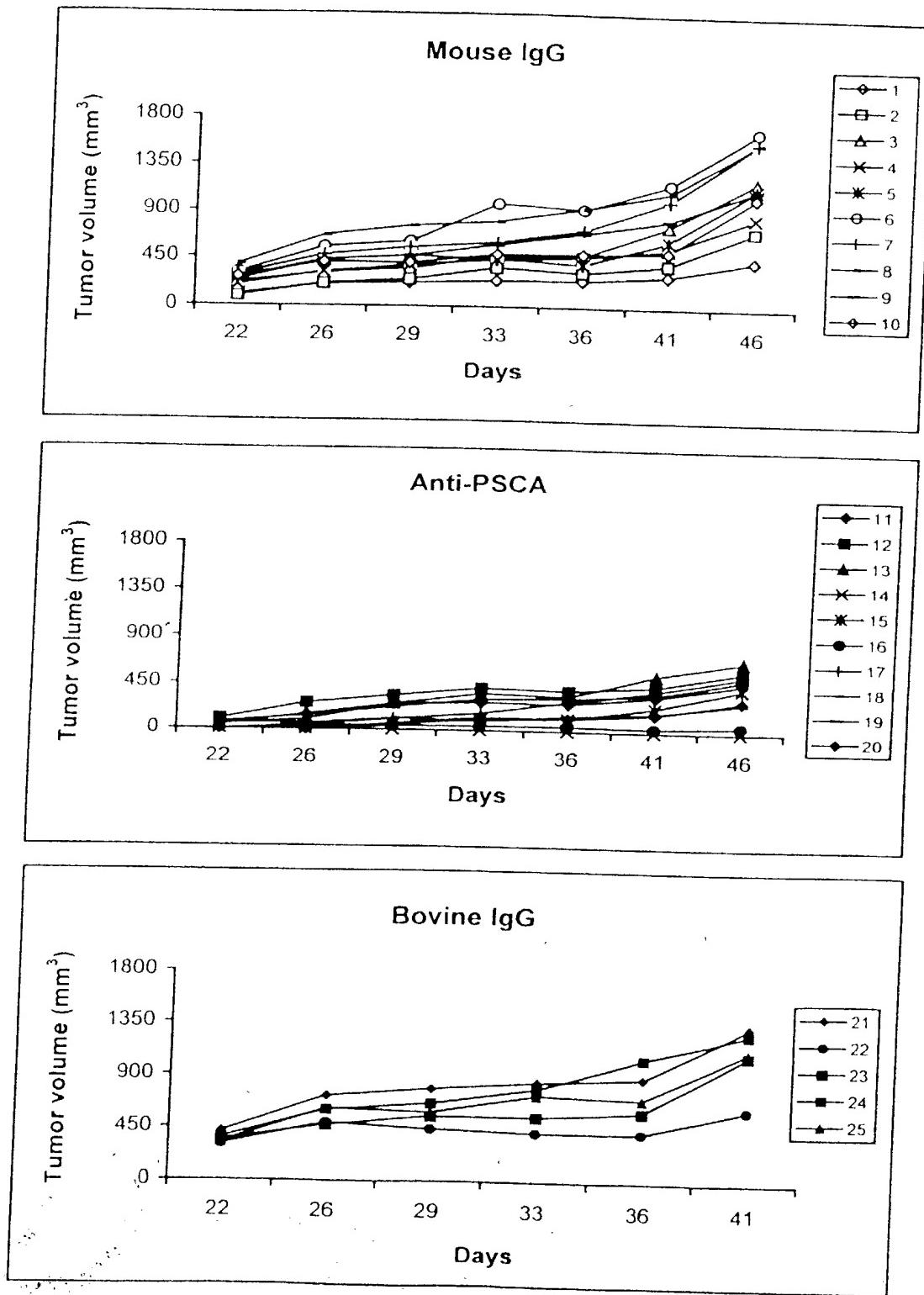


FIG. 54

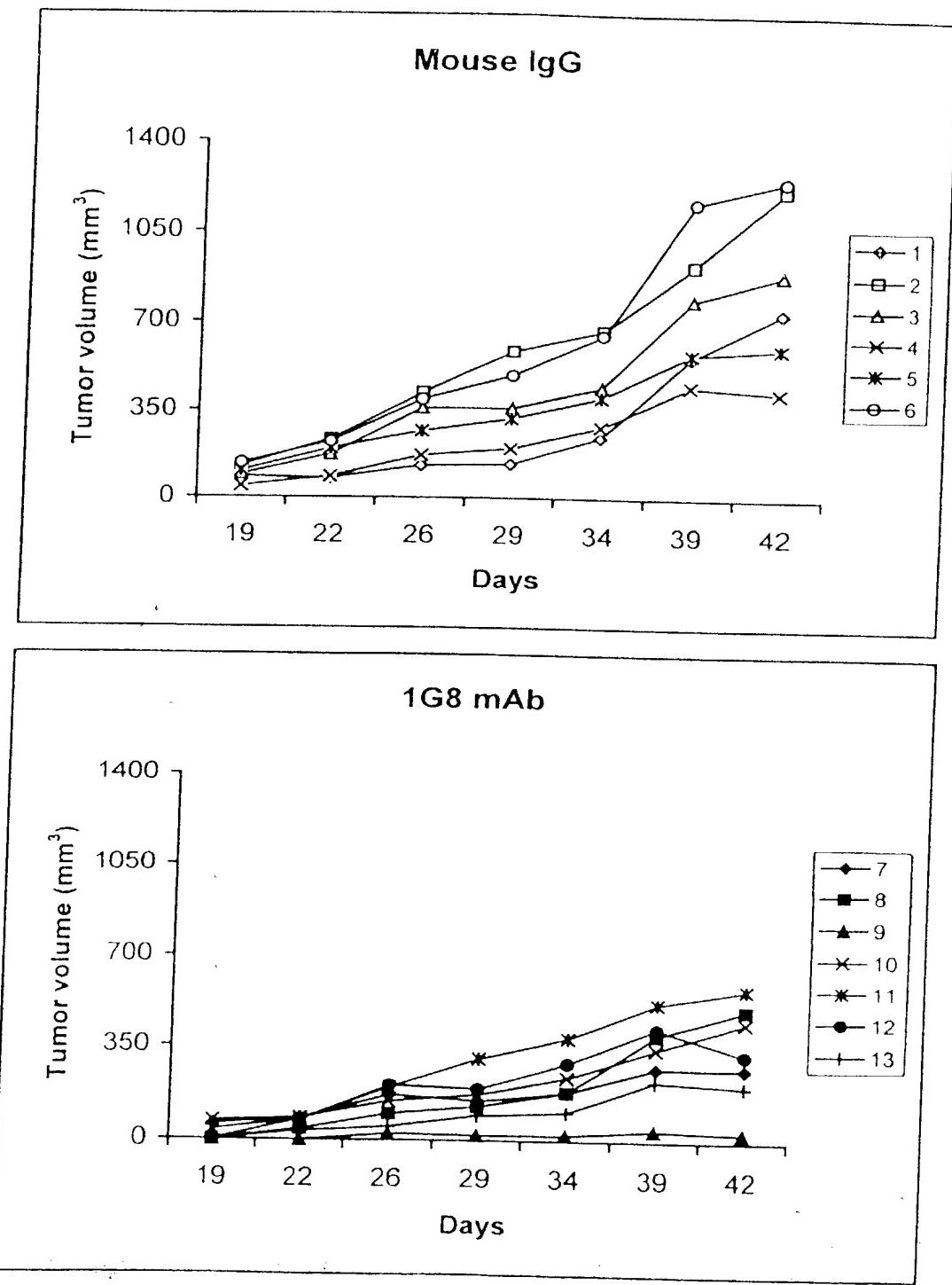


FIG. 55

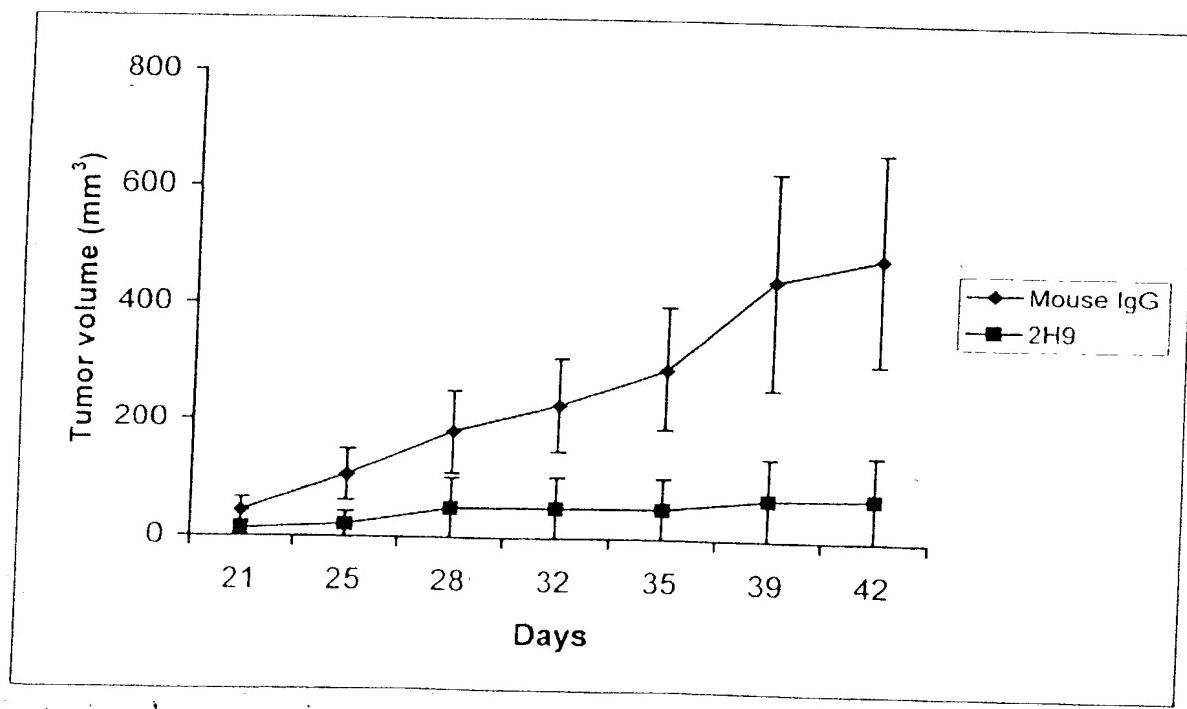
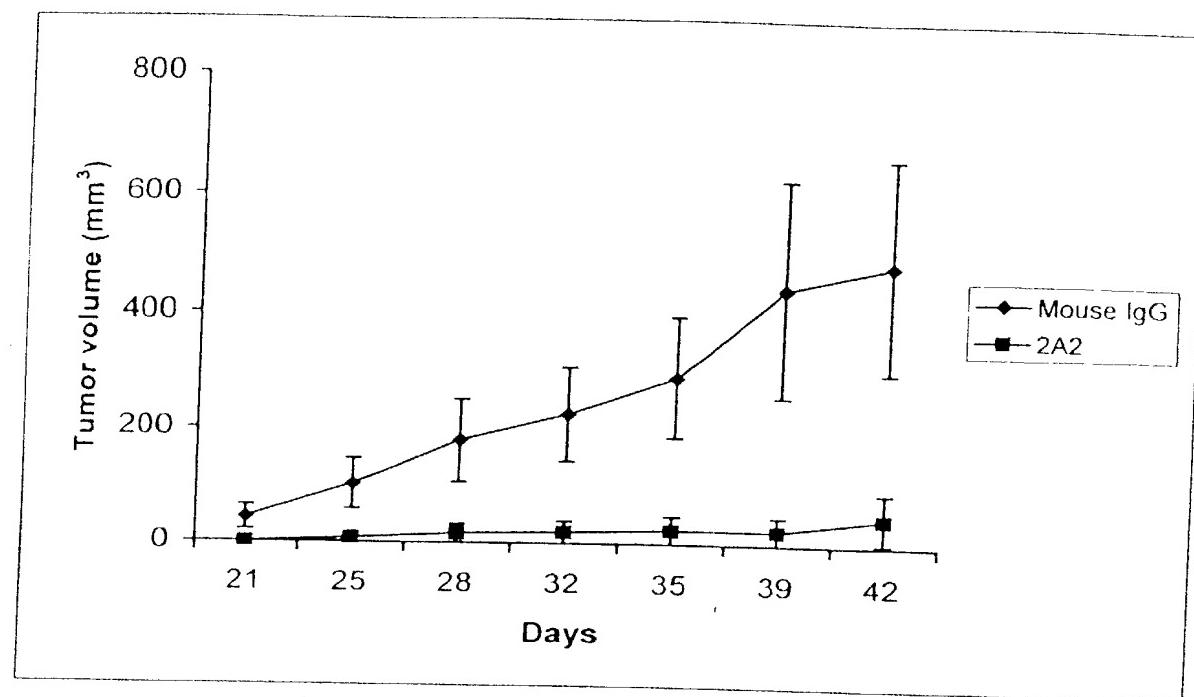


FIG. 56

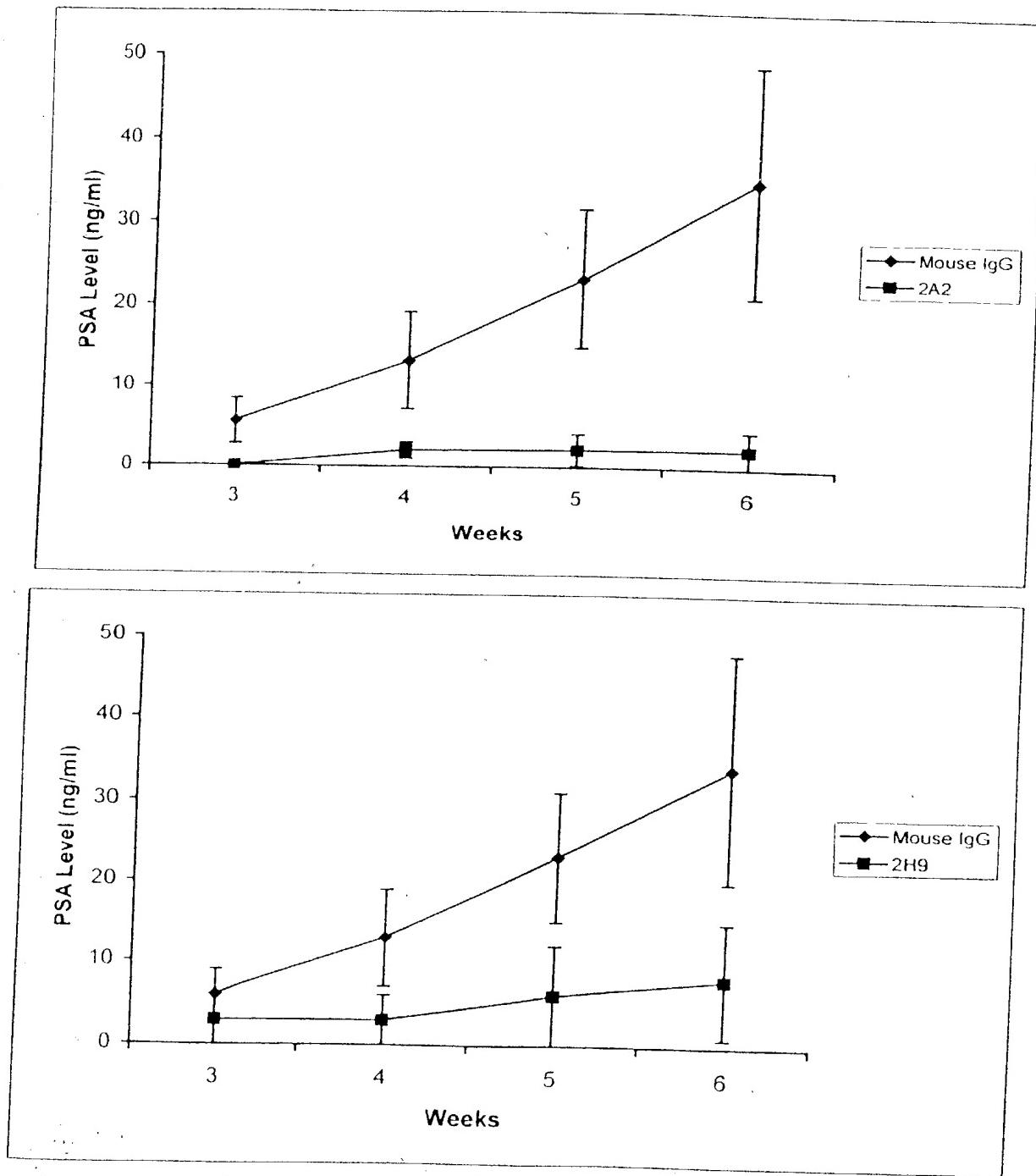


FIG. 57

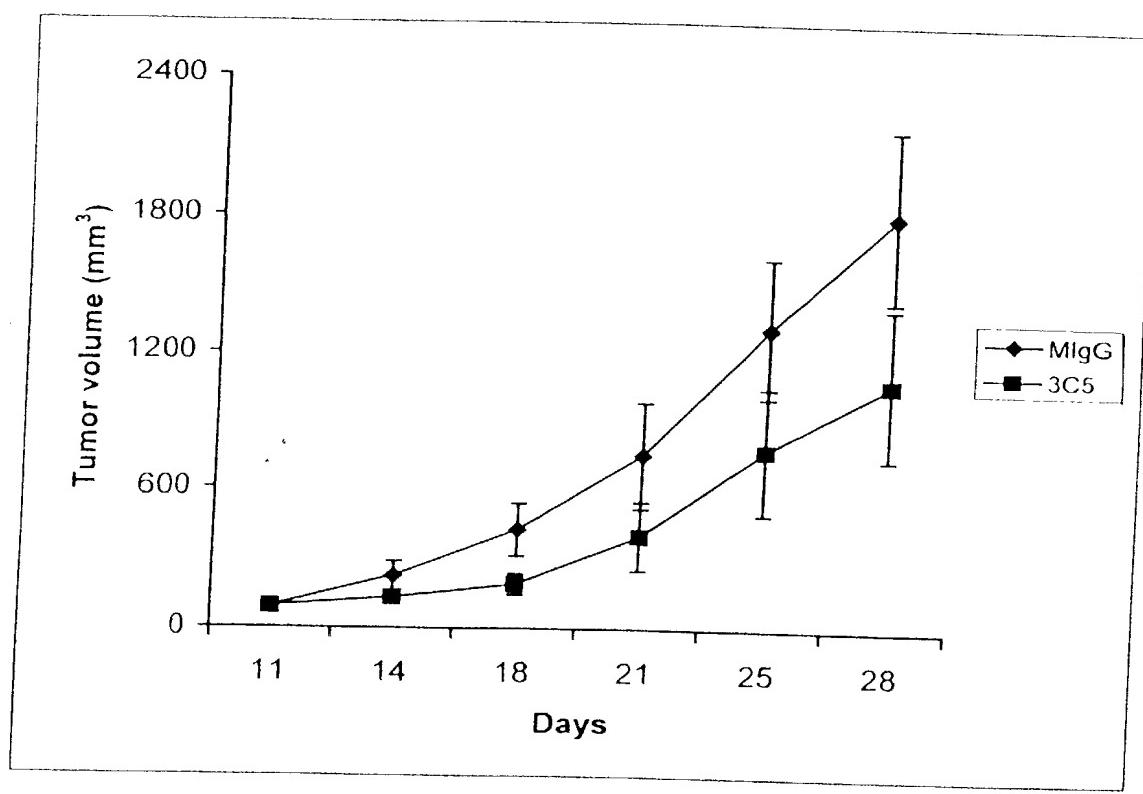


FIG. 58

TGCTTCTTCCTGATGGCAGTGGTTATAGGAGTCATTCAATTCAAGGGTTACAGGCTAGCTGCAGCAGTCT 60
 C F F L M A V V I G V N S E V Q L Q Q S 20

GGGCAGAACTTGTGAGGTCAAGGGCCTCAAGTCAGTGTCCACAGCTTCTGGCTTC 120
 G A E L V R S G A S V K L S C T A S G F 40

CDR1

AACATTAAAGACTACTATAACACTGGGTGAATCAGAGGCCCTGACCAGGGCTGGACTGG 180
N I K D Y Y I H W V N Q R P D Q G L E W 60

CDR2

ATTGGATGGATTGATCCCTGAGAAATGGTGAACACTGAATTGTCCCGAAGTCCAGGGCAAG 240
 I G W I D P E N G D T E F V P K F Q G K 80

GCCACTATGACTGCAGACATTCTCCAAACACAGCCTACCTGCACCTCAGCAGCCTGACA 300
 A T M T A D I F S N T A Y L H L S S L T 100

TCTGAAGACACTGCCGTCTATTACTGTAAAACGGGTTCTGGGCCAAGGGACTCTG 360
 S E D T A V Y Y C K T G G F W G Q G T L 120

GTCACTGTCTCTGCAGCCAAGACACCCCCATCTGTCTATCCACTG
 V T V S A A K T T P P S V Y P L

FIG. 59

TTGGTAGCAAACAGCCTCAGATGTC~~CCACT~~CCCAGGTCCA~~ACT~~GCAGCAACCTGGGTCTGAA 60
L V A T A S D V H S Q V Q L Q Q P G S E 20

CTGGTGAGGCC~~T~~GGAACTTCAGTGAAGCTGGT~~CC~~TGCAAGGCTTCTGGCTATACTCTGCC 120
L V R P G T S V K L S C K A S G Y T F S 40
CDR1

AGCTACTGGATGCCACTGGGTGAAGCAGAGGCC~~T~~GGACAAGGCC~~T~~TGAGTGGATTGGAAAT 180
S Y W M H W V K Q R P G Q G L E W I G N 60

ATTGACCCTGGTAGTGGTTACTAACTAACTAACGCTGAGAACCTCAAGACCAAGGCCACACTG 240
I D P G S G Y T N Y A E N L K T K A T L 80
CDR2

ACTGTAGACACATCCTCCAGCACGCC~~T~~ACATGGCAGCTCAGCAGCC~~T~~GACATCTGAGGAC 300
T V D T S S T A Y M Q L S S L T S E D 100
CDR3

TCTGCAAGTCTATTACTGTACAAGCCGATCTACTATGATTACGACGGATTGCTTACTGG 360
S A V Y C T S R S T M I T T G F A Y W 120

GGCCAAGGGACTCTGGTCACTGTCTGCAGCCTACAAACAGCCCCATCTGTCTATCCA 420
G Q G T L V T V S A A T T A P S V Y P 160

CTGGCC
L A

FIG. 60

AATGACTTCGGGTTGAGCTGGTTTATTATTGTCTTTAAAGGGTCCGGAGTGAA 60
N D F G L S W V F I I V L L K G V R S E 20

GTGAGGCTTGAGGAGCTGGAGGGCTGGCAACCTGGAGATCCATGAACCTCTCC 120
V R L E S G G W V Q P G S M K L S 40

TGTGTAGCCCTCTGGATTACTTCAGTAATTACTGGATGACTTGGCTGCCAGTCTCCA 180
C V A S G F T F S N Y W M I W V R Q S P 60
CDR1

GAGAAGGGCCTGAGTGGGTTGCTGAAATTGAGATCTGAAAATTATGCAACACAT 240
E K G L E W V A E I R L R S E N Y A T H 80
CDR2

TATGGGAGTCTGTGAAAGGGAAATTCAACCATTCTAAGAGATGATTCCAGAAAGTGTCTC 300
Y A E S V K G K F T I S R D D S R S R L 100
CDR3

TACCTGCAAATGAAACACTTAAGACCTGAAAGACAGTGGAAATTATTACTGTACAGATGGT 360
Y L Q M N N L R P E D S G I Y Y C T D G 120

CTGGGACGACCTAACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCAGCCAAACGACA 420
L G R P N W G Q G T L V T V S A A K T T 140
CDR3

CCCCCATCTGTCTATCCACTGGCCCTTGTGTA
P P S V Y P L A P C V

FIG. 61

CDR1 Comparisons

1G8	lgG _{1k}	Middle	G F N I K D Y Y I H
2H9	lgG _{1k}	N-Term.	G F T F S N Y W M T
4A10	lgG _{2ak}	N-Term.	G Y T F S S Y W M H

CDR2 Comparisons

1G8	lgG _{1k}	W I D P E N G D T E F V P K F Q G
2H9	lgG _{1k}	E I R L R S E N Y A T H Y A E S V K G
4A10	lgG _{2ak}	N I D P G S G Y T N Y A E N L K T

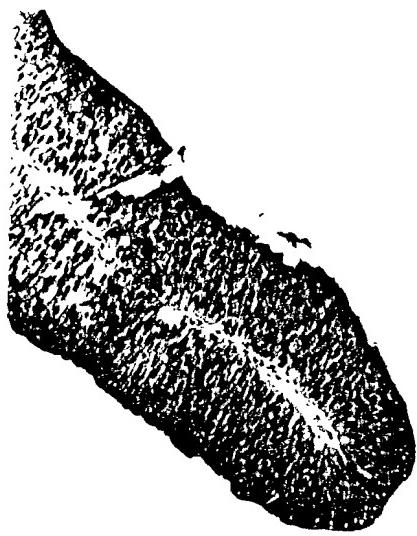
CDR3 Comparisons

1G8	lgG _{1k}	G G F
2H9	lgG _{1k}	L G R P N
4A10	lgG _{2ak}	R S T M I T T G F A Y

FIG. 62



B



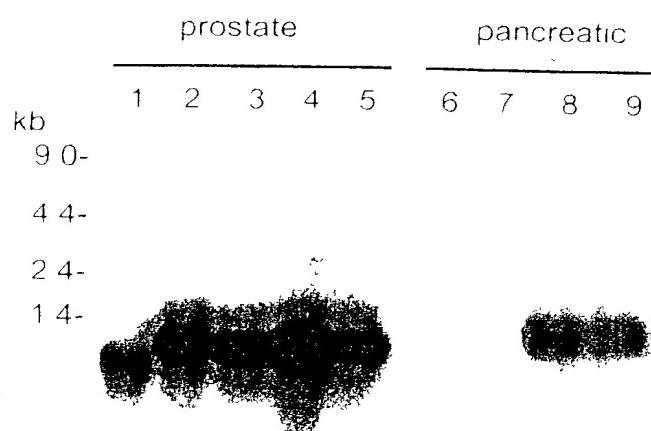
C



D



FIG. 63



- | | |
|--------------|-----------|
| 1. Prostate | 6 PANC-1 |
| 2. LAPC-4 AD | 7 BxPC-3 |
| 3. LAPC-4 AI | 8 HPAC |
| 4. LAPC-9 AD | 9 Capan-1 |
| 5. LAPC-9 AI | |

FIG. 64

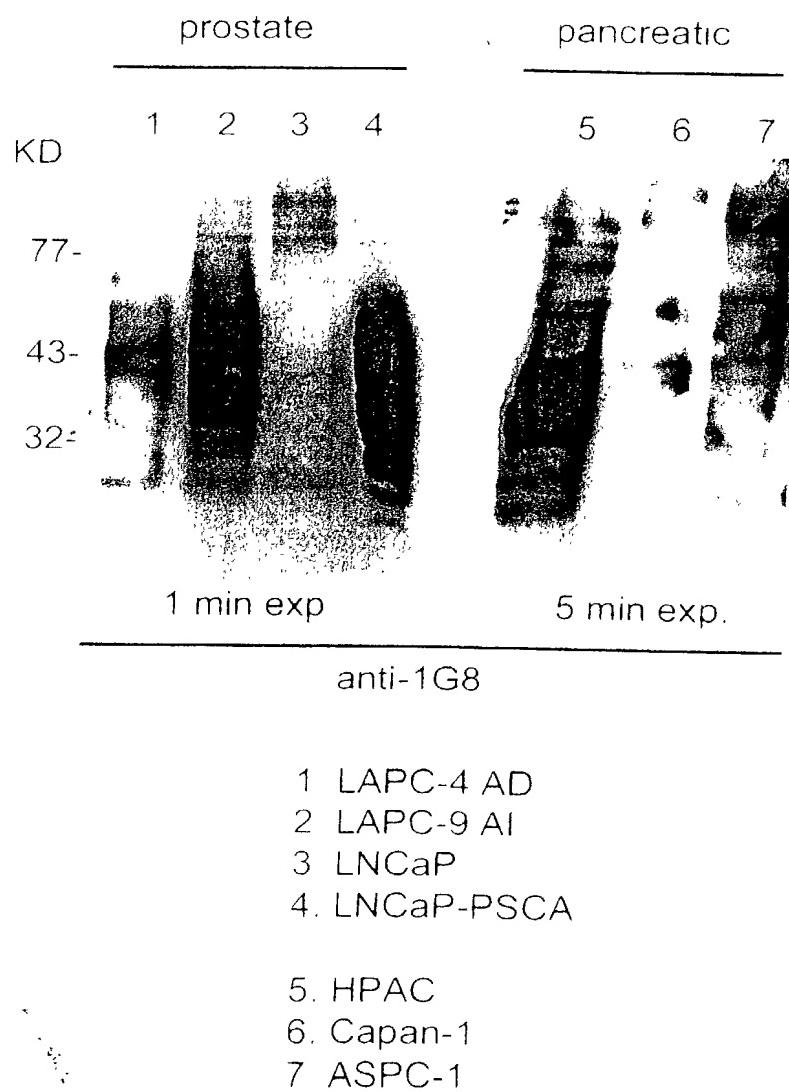
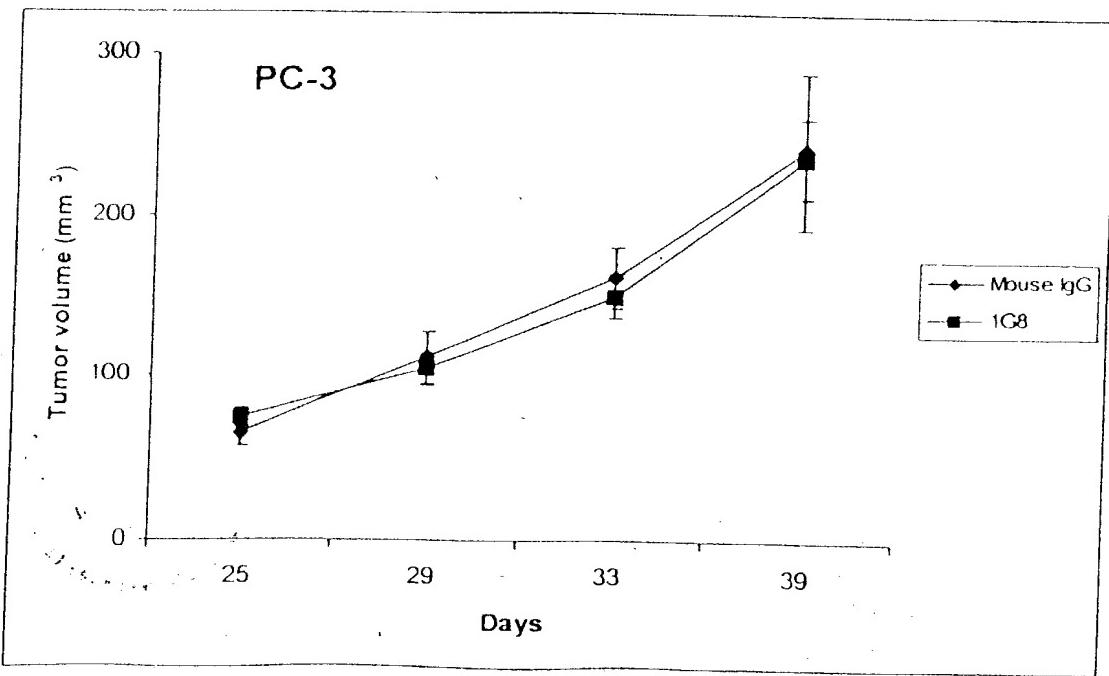
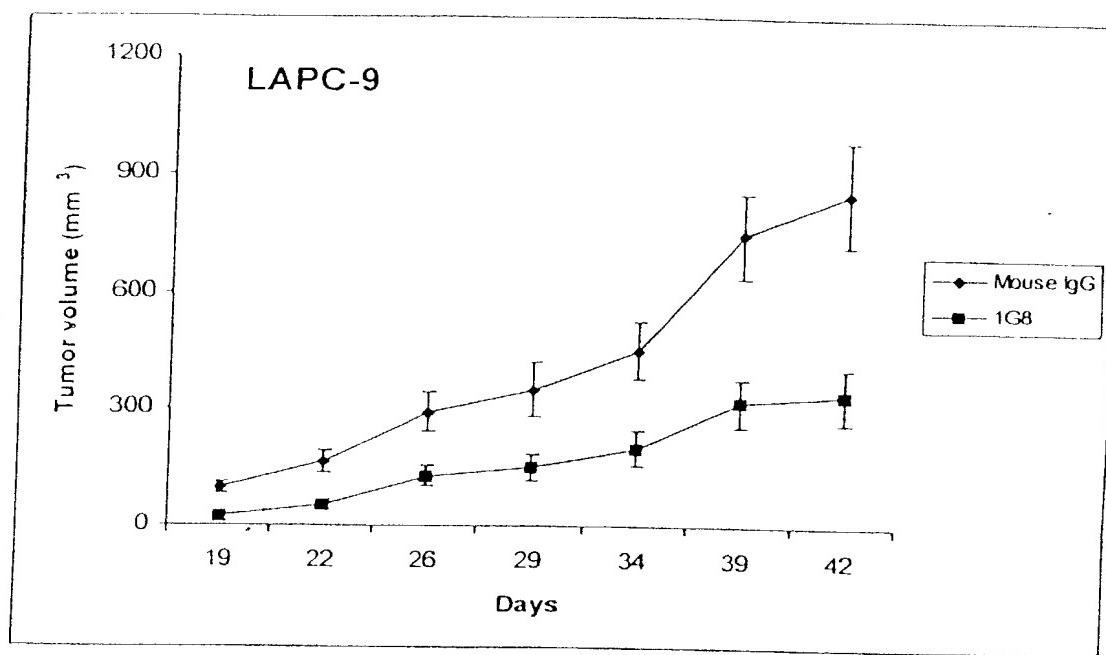
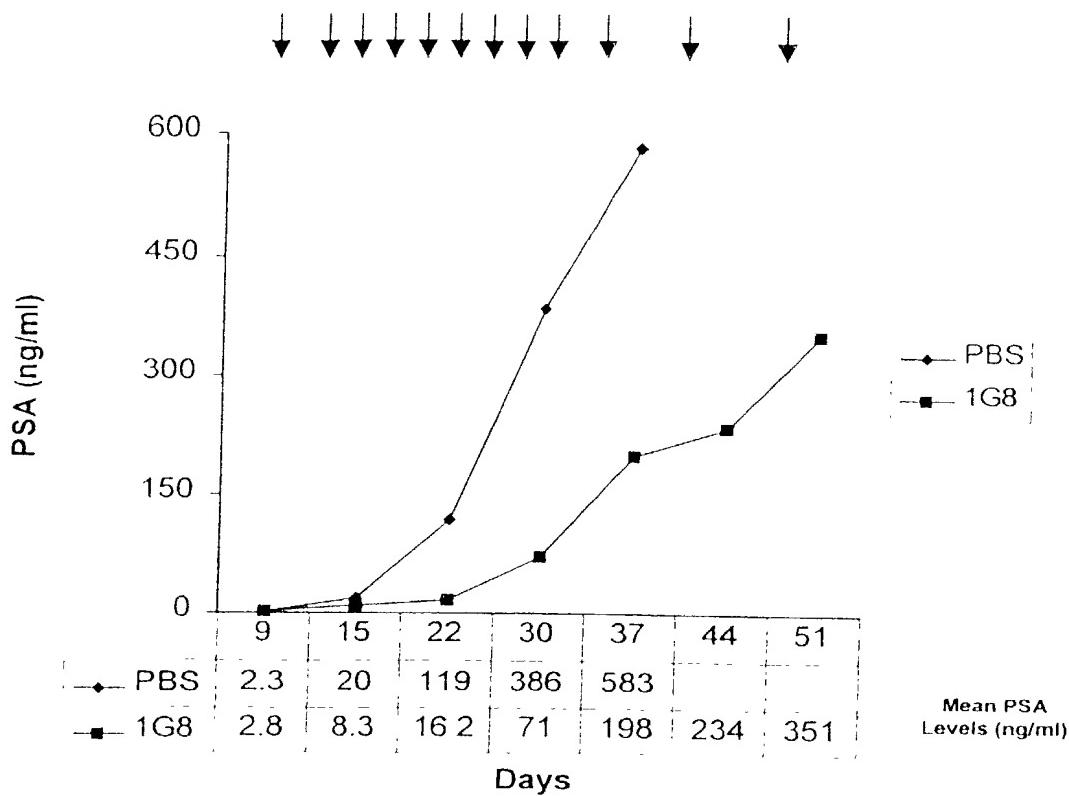


FIG. 65



F I G . 66

A)



B)

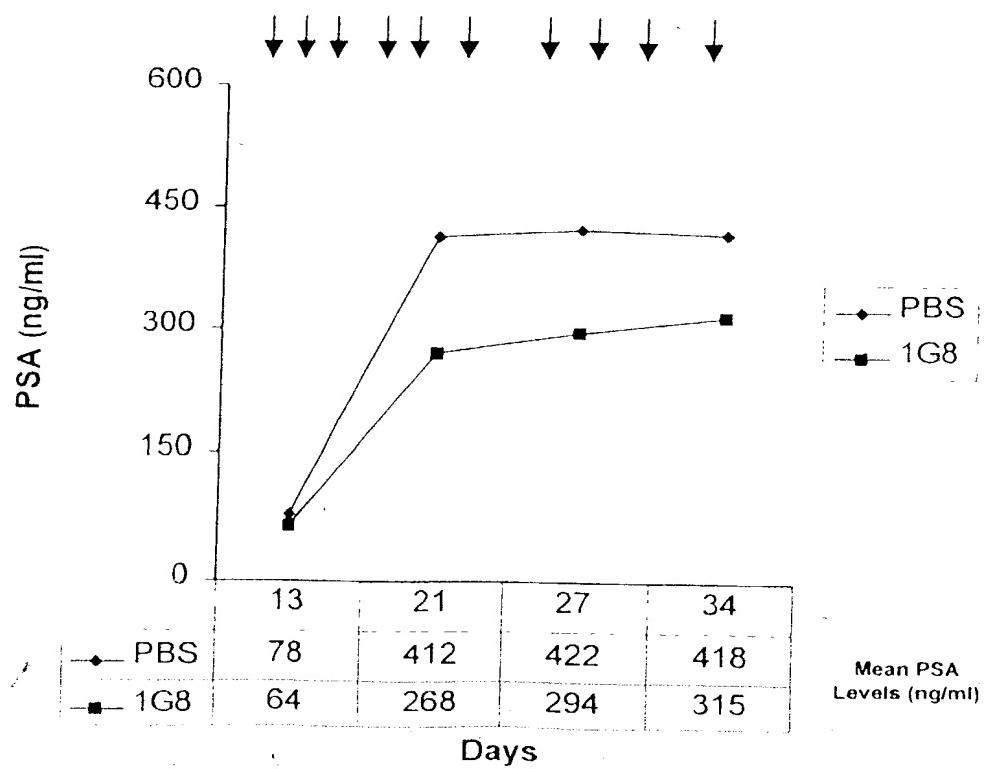
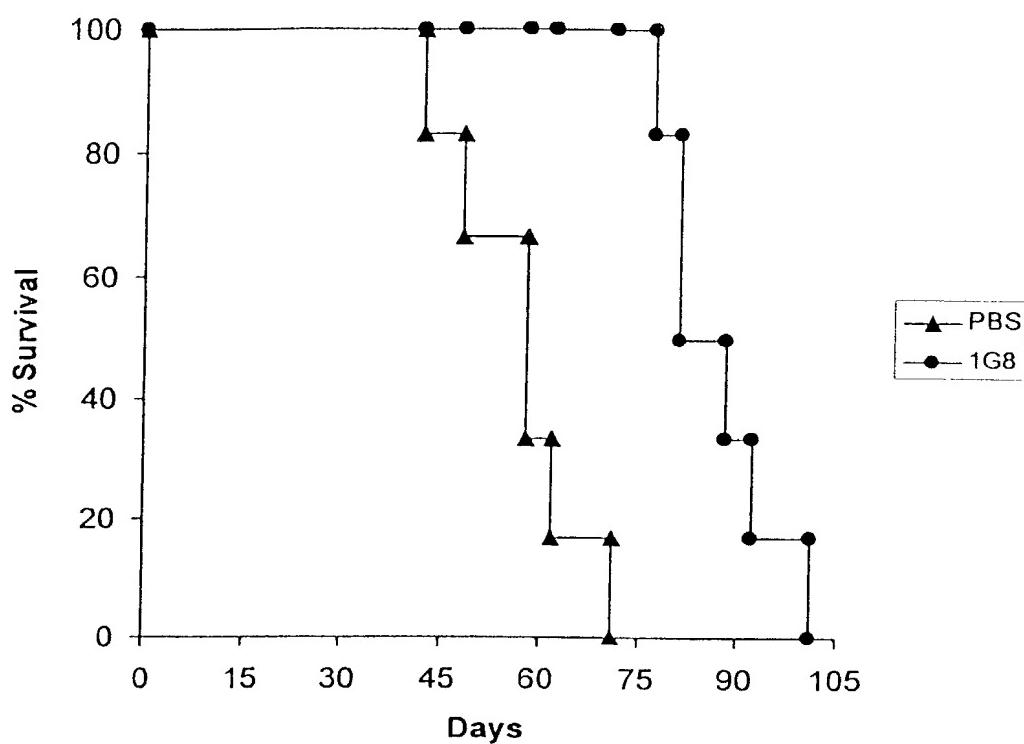


FIG. 67

A)



B)

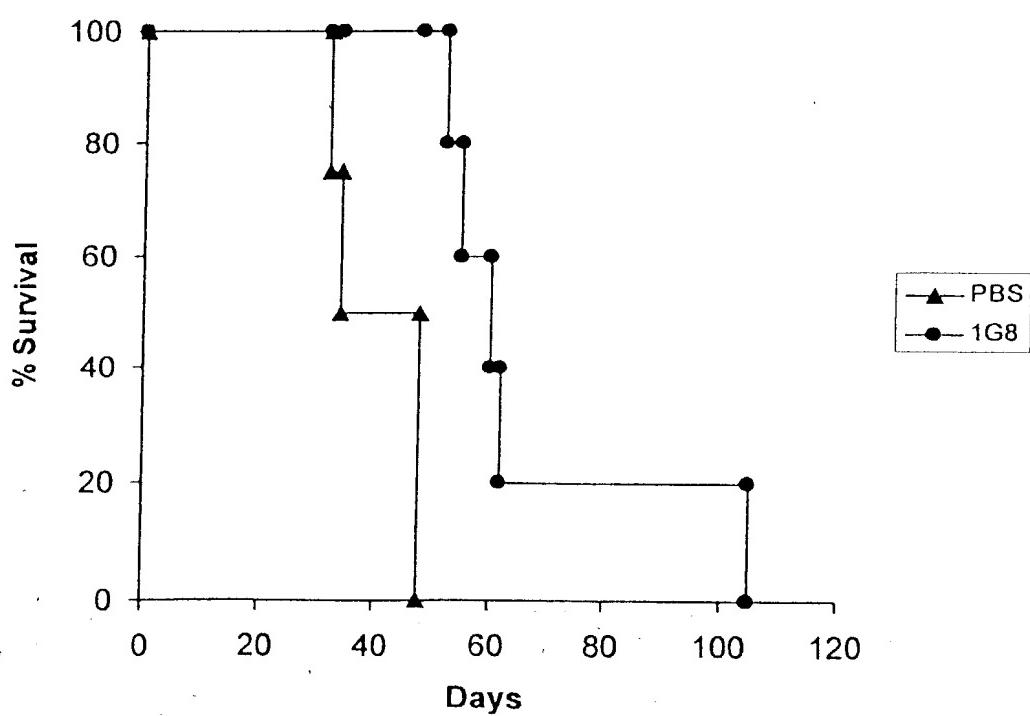
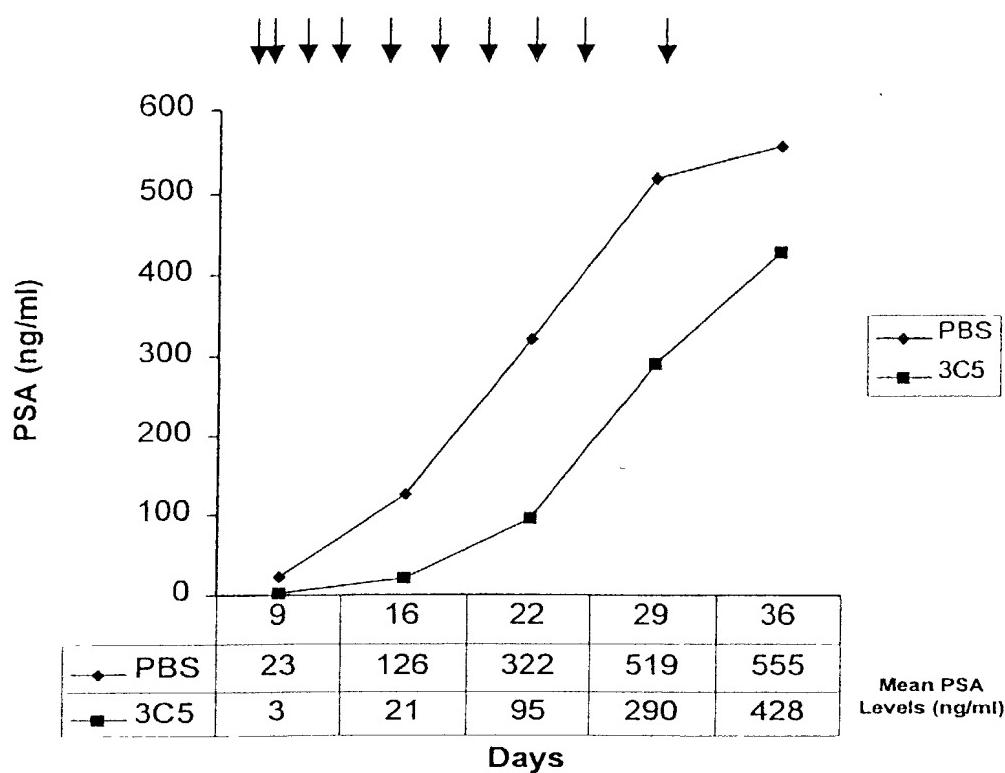


FIG. 68

A)



B)

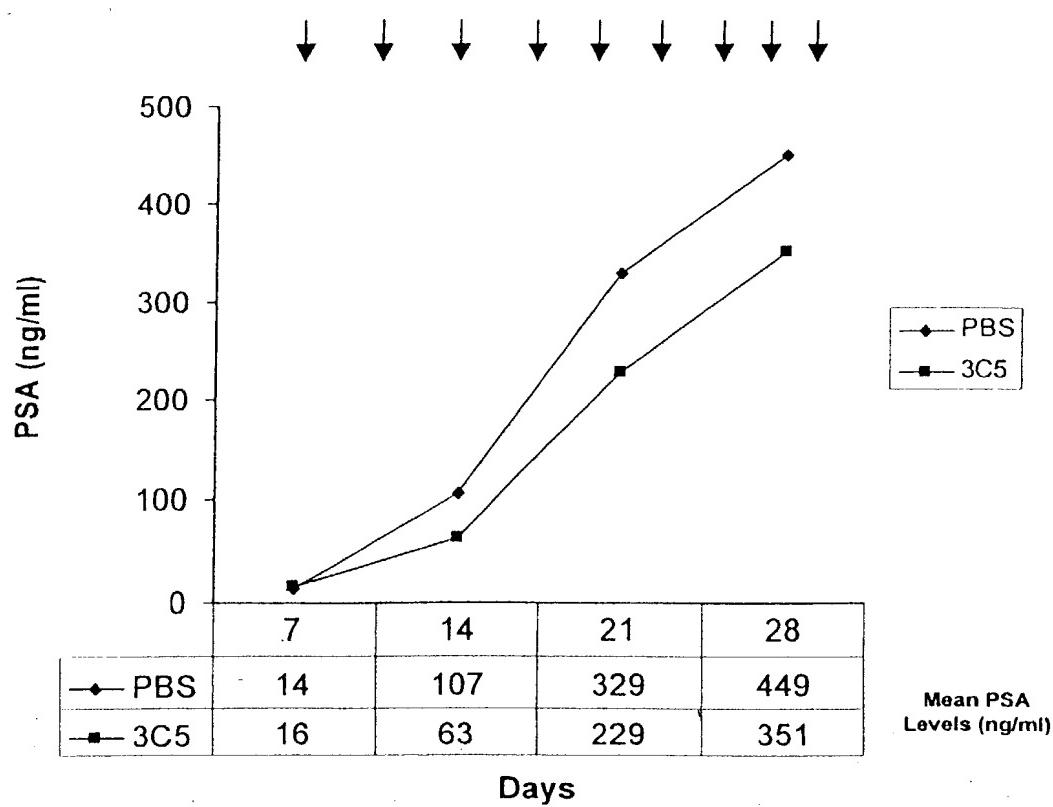
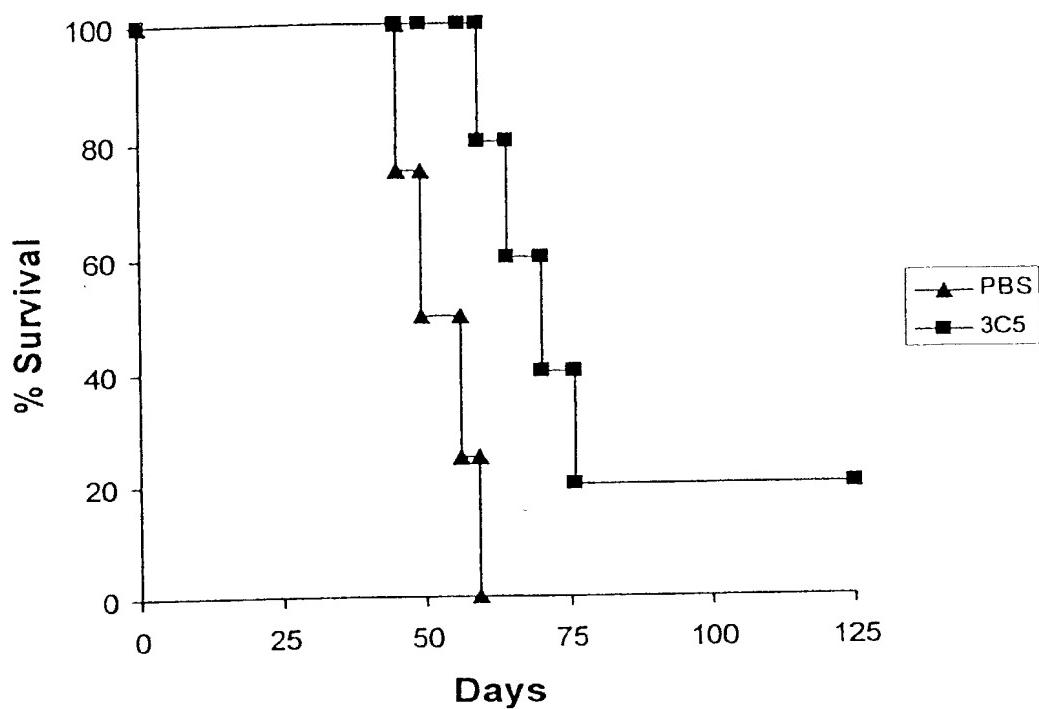


FIG. 69

A)



B)

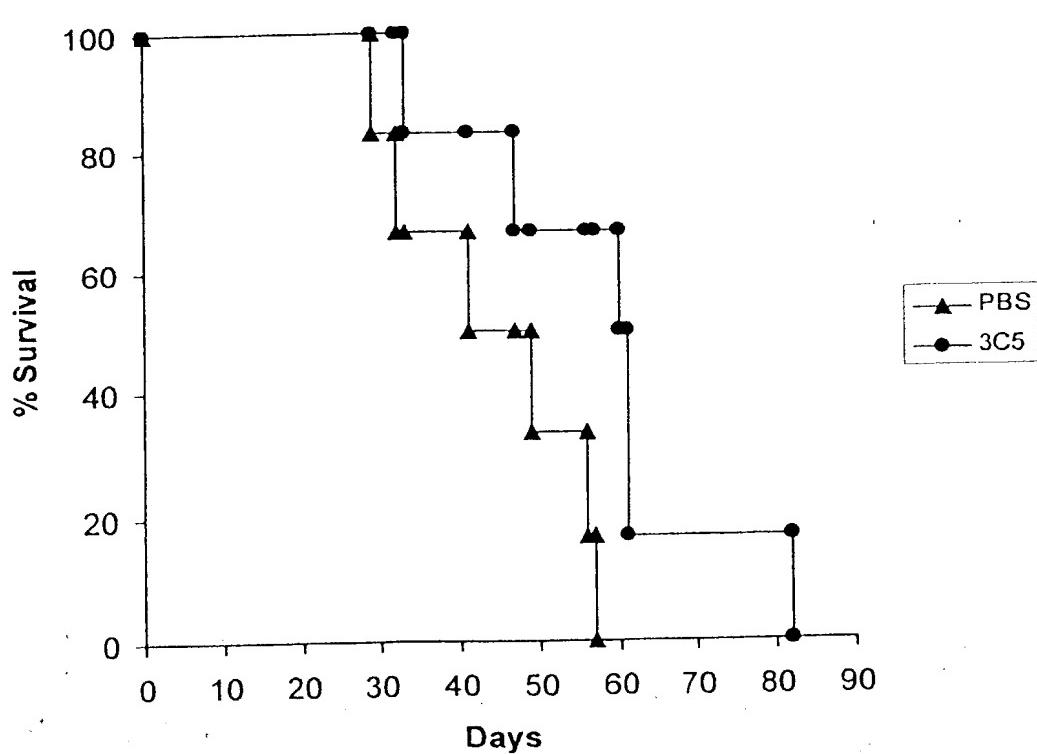


FIG. 70

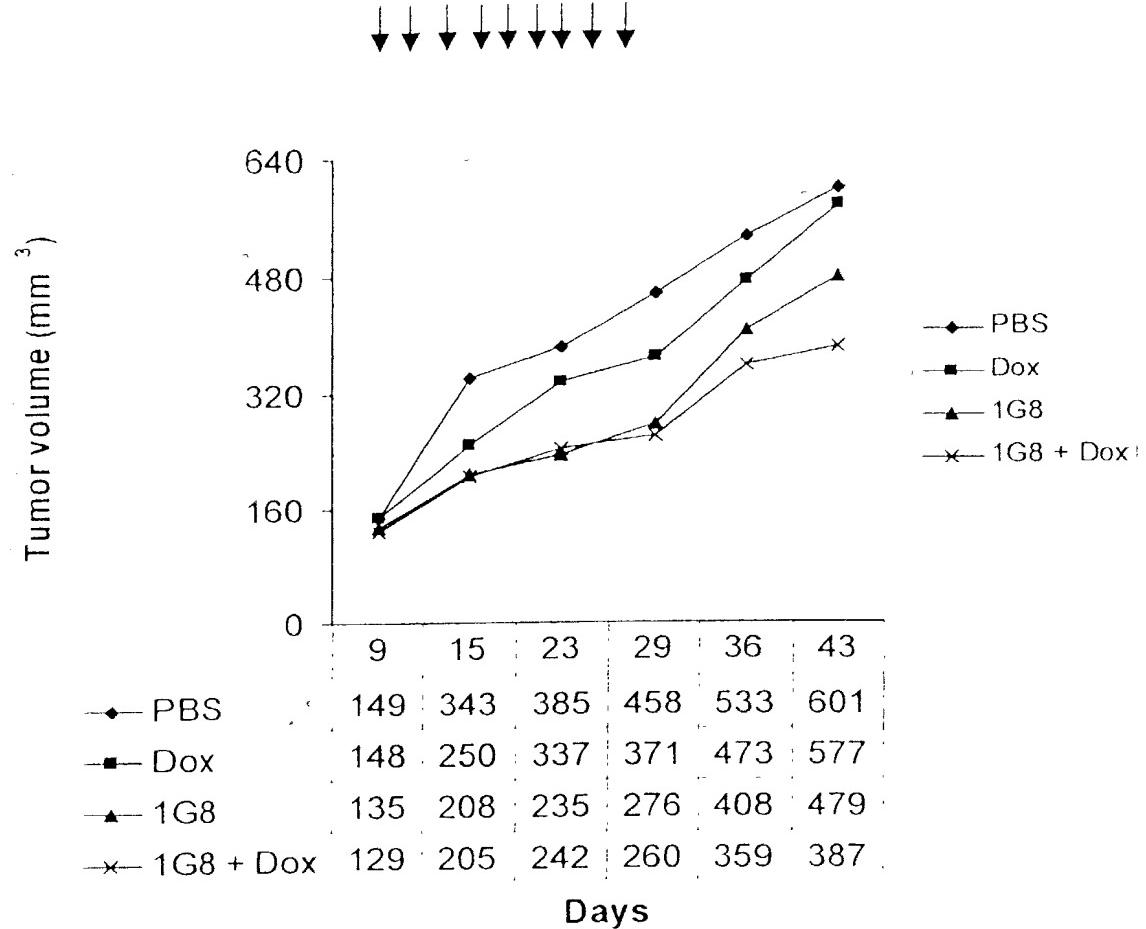
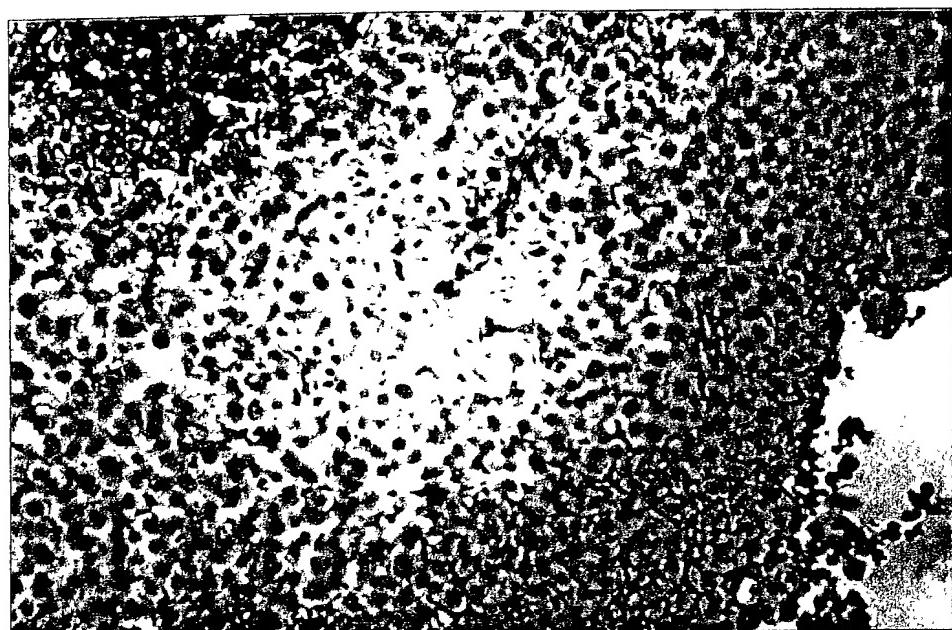


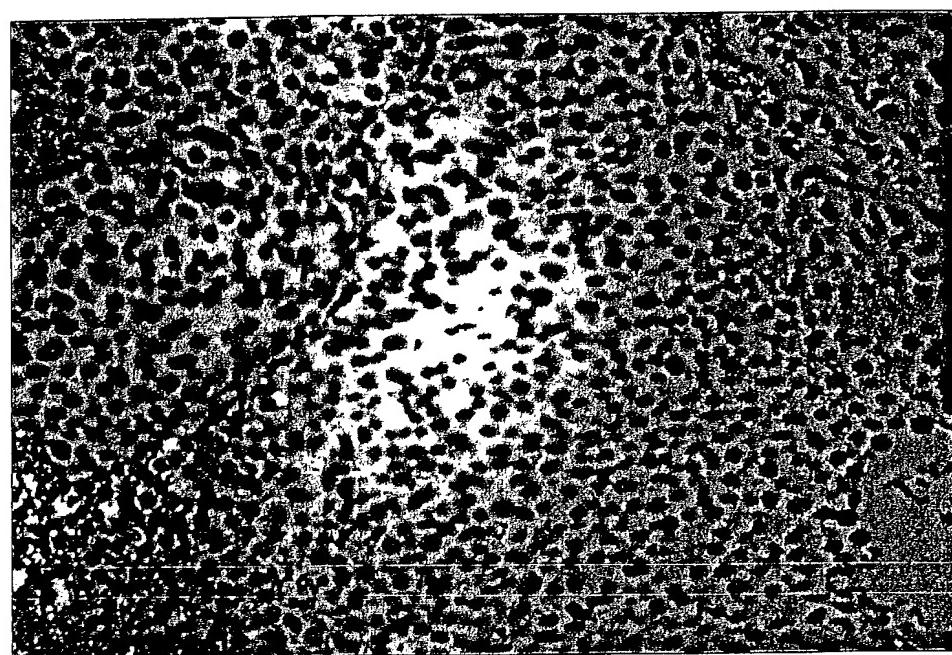
FIG. 71

PSCA 3C5 MAb Localizes within
LAPC9AD Xenograft Tissue

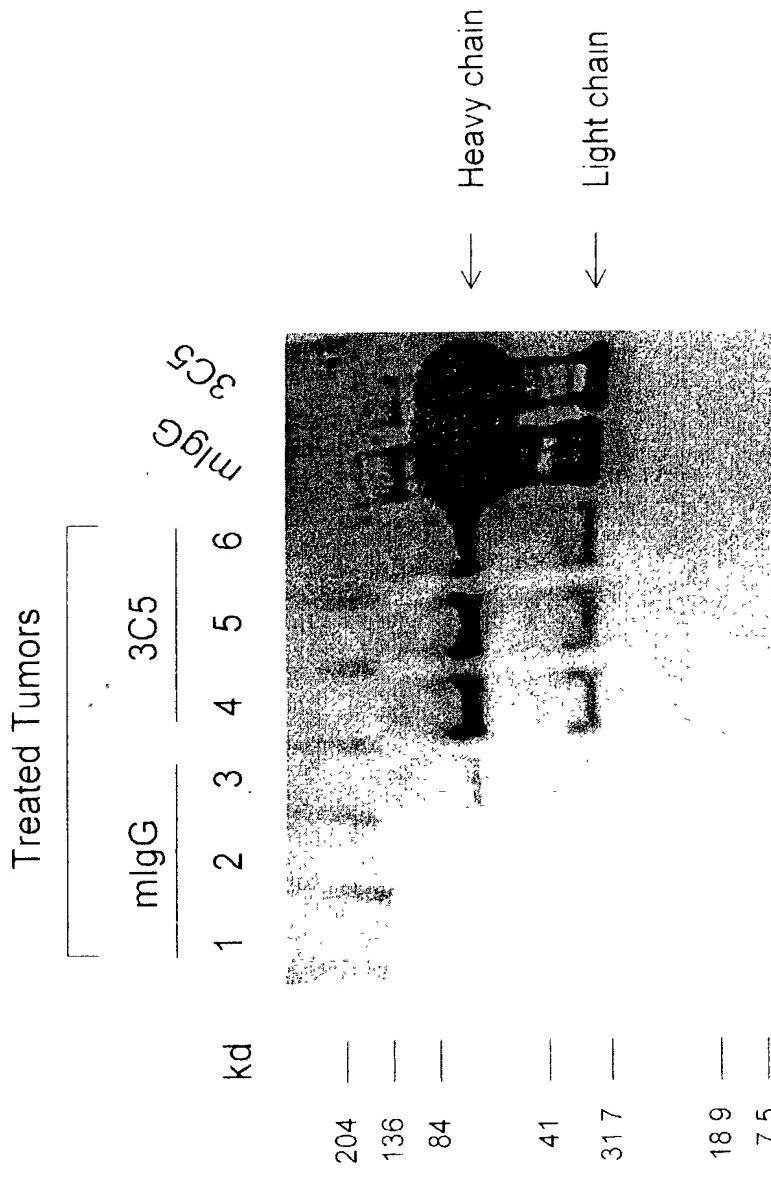
3C5 Treated



mIgG Treated



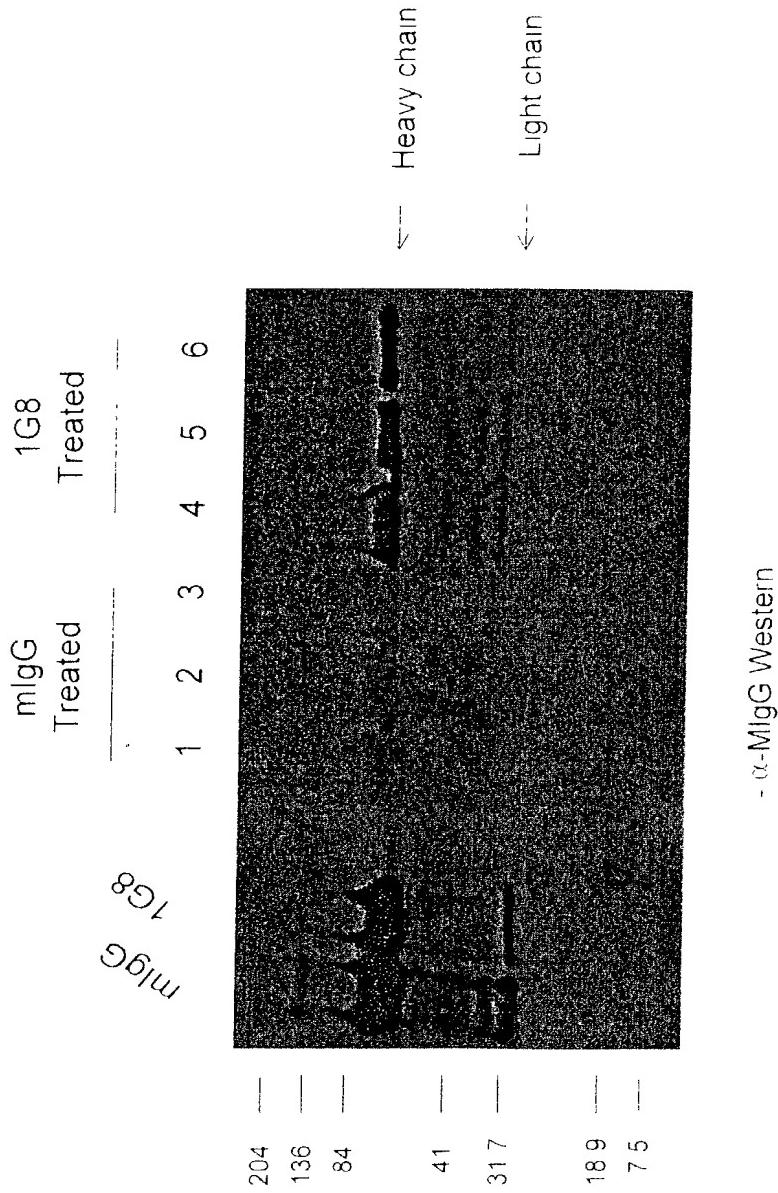
3C5 Anti-PSCA MAb is Localized to Established LAPC-9 Tumors



Western blot developed with $\alpha\text{-mIgG}/\kappa$

FIG. 72

SPECIFIC TARGETING OF THE 1G8 ANTI-PSCA MAb TO ESTABLISHED LAPC-9 TUMORS



Method: Mice bearing established LAPC-9 tumors ($>100 \text{ mm}^3$) were injected with either mIgG or the anti-PSCA MAb 1G8. Tumors were harvested a week later and made into protein lysates for Western analysis.

FIG. 73